Dedication

This Second Edition of the Telomere Biology Disorders Diagnosis and Management Guidelines is dedicated to our community: to our founders, our newly diagnosed, our bereaved, our researchers and clinicians, to all people who have been impacted by TBDs who have paved the way of this journey. These guidelines are made possible because of you.

Dedicated to those who love and watch the suffering and desire to make better the lives of those we love.

Dedicated to those willing to give consent for research that you know may never help you or those we love in real time.

Dedicated to you who wish that no one else would endure as you have had to endure.

Dedicated to the minds willing to explore the possibilities of science.

Dedicated to those who never stop exploring and never stop being curious and never stop trying.

We dedicate these guidelines to a community that has built an unending chain of hope, together.

Our wish is that these guidelines provide life-prolonging guidance, hope for the future, and an affirmation that you are never alone.

Thank You, Donors!

Thank you to each donor in all capacities. From grassroot efforts to corporate sponsorships, we are grateful for those that contributed to make these Guidelines possible.

We thank Repeat Diagnostics Inc. for their generous sponsorship.

This document can be found at https://bit.ly/TTguidelines. A printed copy may be requested by emailing info@teamtelomere.org, or for newly diagnosed families, via our Care Package Program at teamtelomere.org/care-package-program.

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Telomere Biology Disorders
Diagnosis and Management Guidelines
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On behalf of the entire Telomere Biology Disorder community: thank you. Thank you for the time, energy, and effort that you have poured into your chapter for these Guidelines. Your dedication to the patients in our community and to the education of medical professionals is deeply appreciated every day. As telomere science evolves, it is crucial that our team continue to collaborate and grow to best serve all those with telomere biology disorders. Thank you to the founders of Dyskeratosis Congenita Outreach and contributors of the first edition of the clinical guidelines. You are and have been critical to the evolution of Team Telomere. A full list of contributors can be found at the end of these Guidelines.

Table of Contents

Welcome

Dyskeratosis Congenita Outreach, Inc. (DCO) was unofficially born in 2006 when two people affected by this rare, genetic condition connected over the common desire to reach out. Together they created a conduit of support among patients and families that had never before existed.

At its inception, DCO was simply a name, a website, and an email address, yet a much-needed component of the dyskeratosis congenita landscape. The absence of a family support group had also been recognized by physicians involved with the National Cancer Institute's (NCI) Inherited Bone Marrow Failure Syndrome Study. It was a void that a team of scientists, led by Dr. Blanche Alter, principal investigator of the study, set about filling by procuring funding to sponsor a family symposium for DC patients. Held in Bethesda, Maryland, in September 2008, the meeting allowed DC families to meet for the first time. Some of them had met informally through the DCO website, but most were coming face to face with another DC patient for the first time. The meeting attendees, who likely would not have met otherwise, bonded over shared experiences. A number of them quickly organized into a group driven to expand the DC community.

Fast forward 16 years, to where we are now. DCO laid the foundation for what would become Team Telomere, Inc., an international community for Telomere Biology Disorders (TBDs). Although outreach was the original framework for the organization, it quickly grew to become a support for families and a hub for research, as well as the leading organization worldwide to support and educate every stakeholder in the TBD community.

To date, Team Telomere has given away thousands of first edition clinical guidelines and 300 care packages to patients in 22 countries. Team Telomere has fundraised over $400,000 through our annual Million Dollar Bike Ride and has representation in nine countries. In 2021 Team Telomere was selected from over 200 applicants as one of the recipients of the prestigious $600,000 Rare as One grants from the Chan Zuckerberg Initiative. All of our work over the years has been in support of the foundational mantra of one of Team Telomere's founders, Nancy Cornelius: "You are never alone."

Just as Team Telomere's organization name and focus have evolved over the years, so has our understanding of the underlying disease that unites us: telomere biology disorders. Dyskeratosis congenita is one TBD that will always be a focus for Team Telomere, but many in our community lack the classical mucocutaneous triad characteristic of dyskeratosis congenita and thus do not identify with this terminology. Our patient population spans the very young to the very old and all genders. As evidenced by the length and breadth of these guidelines, virtually any combination of body systems may be affected by this disease, or none at all in some patients. Despite the myriad manifestations, what unites us all lies in our chromosomes. To bridge the gap between our heritage as DCO and our future as Team Telomere, we have chosen to refer to our disease as "DC/TBD" in these guidelines. Although some areas of these guidelines make reference to one or more specific TBDs, our goal is to embrace and provide comprehensive information about all TBDs identified to date and TBDs in general.

Team Telomere is proud to present to you the second edition of Telomere Biology Disorders Diagnosis and Management Guidelines. Our sincere and deep gratitude goes to all those that have come before us to pave the way in science and advocacy. Thank you to our Team Telomere families; we honor your journey and are humbled to walk with you throughout. We maintain that you are never alone.

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Chapter 1

Introduction

An Introduction to the Second Edition
of the Telomere Biology Disorders
Diagnosis and Management Guidelines

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It is with both great pride and humility that I introduce you to the second edition of the Telomere Biology Disorders Diagnosis and Management Guidelines. The pride stems from watching Team Telomere grow from the fledgling group known as Dyskeratosis Congenita Outreach to a global advocacy, education, and outreach organization. My humility as an editor of the second edition and a telomere biology disorder researcher comes from the recognition that our discoveries of the clinical manifestations and genetic causes of these disorders are just the beginning.

Dyskeratosis congenita (DC) was first described between 1906 and 1910 [1]. The initial reports suggested X-linked recessive inheritance since only males were affected. However, the first female case was published in 1963, and we now know that both males and females can be affected with DC [2]. A slight male predominance remains due to pathogenic variants (mutations) in the dyskerin (*DKC1*) gene located on the X chromosome. Dr. Inderjeet Dokal's research group identified mutations in *DKC1* as the cause of X-linked DC in 1998 [3]. Basic science studies showed that *DKC1* mutations led to abnormal dyskerin protein, very short telomeres, and low levels of telomerase in

patient cells [4]. These studies were the very first to link telomere biology to inherited disease.

Subsequent studies included sequencing genes encoding components of the telomerase enzyme complex in families with DC and the development of leukocyte telomere length measurement by flow cytometry with fluorescent *in situ* hybridization (flow FISH) as the diagnostic test for DC [5-9]. Telomere length, instead of complex clinical manifestations, was key to the discovery of mutations in *TINF2* as a cause of DC [10]. Around the same time, researchers also found mutations in telomere biology genes in families with apparently isolated pulmonary fibrosis [11, 12]. The 2003 completion of the first draft DNA sequence of the human genome led to a genomic revolution, making it possible and affordable to effectively and efficiently identify the genetic causes of many disorders. Pathogenic variants (*i.e.,* germline mutations) in at least 15 different telomere biology genes have since been identified and associated with a spectrum of manifestations, from very early childhood to adult-onset illnesses associated with telomeres ranging from significantly less than the 1st percentile to the 10th percentile for age [13, 14].

These breakthroughs over the last decade led to a growing appreciation of the clinical spectrum of illnesses now called the telomere biology disorders (TBDs). Some people have childhood onset of bone marrow failure, physical manifestations, and many other complications due to X-linked or autosomal recessive pathogenic variants, whereas others may not manifest a TBD until they develop pulmonary fibrosis in middle age. Telomere lengths are also associated with the mode of inheritance and clinical manifestations [7, 15-17]. Connecting these rare disorders through biology helps ensure that a breakthrough, for example, in pulmonary fibrosis due to a heterozygous pathogenic *TERT* variant could also potentially benefit a patient with DC due to biallelic pathogenic *TERT* variants.

These clinical care guidelines are the result of a multi-disciplinary international collaboration between clinicians and scientists united by the goal of improving the lives

of people with TBDs. The clinical management recommendations are based on expert opinions of the chapter authors, reviewers, and editors. While there are ongoing hematopoietic cell transplantation and danazol treatment trials, there are currently no evidence-based TBD-specific clinical management guidelines. It is very important that the care of each person affected by a TBD be multi-disciplinary and specifically tailored to their needs.

So, what is in a name and especially in a long name, Telomere Biology Disorder, with an abbreviation – TBD – that can mean other things (to be determined)? It comes down to their root cause: abnormalities in telomere biology resulting in a spectrum of overlapping illnesses. Understanding the causes and complications of TBDs is the first step. Collaboration between research, clinical, and patient communities will ensure there are many future next steps aimed at improving the lives of all those affected by TBDs.

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Chapter 2

Why Telomeres Matter

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Introduction

Biologists have studied telomeres for many decades — from long before their connection with human disease was known. This chapter provides a background for the chapters that follow by summarizing information obtained from telomere research. It begins with an overview of a few key aspects of biology required to understand what telomeres are, how they function, and the processes that protect and maintain them, and it then describes how defects in these processes can cause disease.

Types of Cells

Organisms are composed of microscopic building blocks called cells and products (like the minerals and proteins that help form bone) that cells export into their environment. Each human contains many trillions of cells.

In the adult body, there are many types of cells with specialized roles, and many different shapes and sizes to suit their functions. One example is peripheral nerve cells, which are very long so they can carry electrical impulses from the spinal cord to a muscle. Another is red blood cells, which are essentially small bags containing the oxygen-binding protein hemoglobin that get pumped around the body inside blood vessels, carrying oxygen from the lungs to other tissues.

One fundamental distinction between cell types relates to whether they are designed to pass on genetic information to the next generation or not. Ova and sperm, the cells that fuse to form a zygote and start the process of embryo formation, and the specialized cells that give rise to ova and sperm, are referred to as germline cells. All other cells in the body are called somatic cells.

Why Cells Need to Make Copies of Themselves

Cells replicate themselves via a process which usually involves growing in size and then dividing into two cells. The process is known as cell division, which means that this is one situation where division is the same as multiplication!

Throughout our lifetime, our cells need to replicate themselves a large number of times. We start off as a single fertilized egg cell (zygote), which needs to undergo a very large number of cell divisions to produce a fully formed baby. Growth of a baby into an adult also occurs through the production of enormous numbers of new cells.

Further increasing the need for cell replication, most types of cells are replaced — in some cases many times over — during a normal life span. There are some exceptions, like certain types of nerve cells that are made early on in embryonic development and then can last for a lifetime, but cells of many other types are broken down and replaced by new ones.

Some of our cells are replaced regularly, which may be thought of as "programmed maintenance." One example is red blood cells, which are replaced with new cells every four months on average; the old ones are broken down and their molecular components are recycled.

Another example is skin cells, which are programmed to die off and shed; they are continuously replaced by new cells. The lining of the bowel is also being continuously replaced. Other types of cells are either replaced when they are damaged or are produced to meet a particular demand. For example, certain types of white blood cells are produced in large numbers when they are needed to fight an infection, and then mostly die off when their job is completed.

The Molecules of Life

Most cells contain a complete copy of the entire instruction set required for them to function and interact with each other. This is encoded in a very long molecule — DNA (deoxyribonucleic acid). In contrast to the binary code used for computers (which consists of long strings of ones and zeros), DNA contains four component nucleotides represented by the letters A (adenine), C (cytosine), G (guanine), and T (thymine), which constitute a four-letter code. The complete set of DNA nucleotides is called the genome, and the DNA of the human genome is divided into 46 pieces called chromosomes. Twenty-three of these chromosomes come from one parent, and the other 23 from the other parent (see Chapter 5, Genetic Counseling for Families). Twenty-two chromosomes are paired (essentially slightly different versions of the same genetic

information) and referred to as autosomes; the remaining two chromosomes are the sex chromosomes, X and Y, which are similar in females (XX), but not in males (XY).

There are many other types of molecules within a living cell, but here just two other types will be mentioned: proteins and RNA (ribonucleic acid) molecules.

Proteins are particularly important because they form much of the intricate machinery that carries out a huge range of processes required for life, including copying DNA and other molecules when cells are getting ready to divide. The precise makeup of individual proteins is encoded within specific regions (genes) of the DNA. Genes are used as the templates for production of RNA molecules, which are transported to areas within the cell responsible for manufacturing proteins (Figure 1).

Therefore, RNA acts as an intermediary molecule, taking instructions from the genome to the places where proteins are made. Biologists refer to the process of making RNA from the DNA code as transcription, and the process of making proteins according to the instructions in the RNA as translation. There are two functioning copies (alleles) of most genes, with many of the genes on the sex chromosomes being notable exceptions.
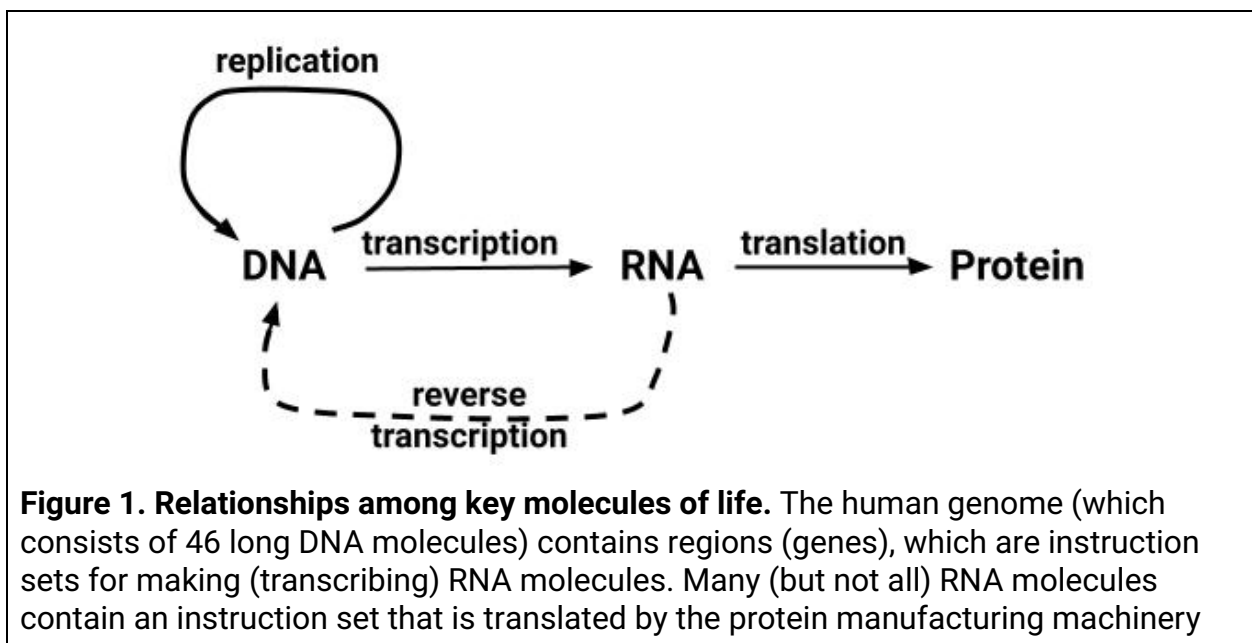
Figure 1. Relationships among key molecules of life. The human genome (which consists of 46 long DNA molecules) contains regions (genes), which are instruction sets for making (transcribing) RNA molecules. Many (but not all) RNA molecules contain an instruction set that is translated by the protein manufacturing machinery

into a specific protein. Specialized proteins and RNA molecules assemble into large molecular machines that make a replicate copy of a cell's DNA (using the existing DNA as a template) when it is getting ready to divide into two cells. Sometimes, an RNA molecule is used as the template for making relatively small pieces of DNA, a process known as reverse transcription.

The way DNA gets copied when cells are being replicated is particularly interesting. DNA has two side-by-side strands, twisted to form the well-known double helix structure discovered by Watson and Crick. The strands are not identical; instead, they are complementary to each other. The relationship between the two strands in the double helix is determined by a very simple rule: wherever one strand has an A the other strand must have a T (and vice versa), and wherever one strand has a C the other strand must have a G (and vice versa).

To copy DNA, specialized proteins pull the strands apart and synthesize a new, complementary strand on each of the existing strands. This means that each DNA copy contains one pre-existing strand plus one newly synthesized strand.

Because RNA molecules are made according to the DNA code (but with different chemical building blocks), they can sometimes be "read backwards" by other specialized proteins to synthesize DNA. This process is called reverse transcription.

Telomeres

The arrangement of the human genome as a collection of 46 chromosomes, each with two ends, presents two big challenges that cells need to deal with. The first challenge is that the DNA copying machinery is incapable of copying all the way to the ends of a DNA molecule. The consequence is that chromosomes get slightly shorter every time cells are replicated.

The second is that cells need to be able to distinguish these 92 ends from accidental breaks elsewhere in the genome. Breaks in DNA have potentially serious consequences

for cells, and cells therefore have elaborate sets of machinery for rejoining fractured ends. It is very important, however, that ends of different chromosomes do not get "repaired" by being inappropriately joined together. This would result in one larger chromosome, which is prone to breakage at a random location, destabilizing the genome.

Cells solve these two problems in the following ways. First, there is a specific DNA code at every chromosome end, which consists of a string of six letters (TTAGGG) repeated many hundreds of times (Figure 2). Because this repetitive DNA does not code for a protein, it is partly dispensable: some of it can be lost due to normal shortening of chromosome ends without adverse consequences.

Figure 2. The DNA code at each chromosome end. One strand is a string consisting of hundreds of copies of the letters TTAGGG. The other strand contains the complementary letters, AATCCC.

Second, there are specialized proteins within the cell that recognize DNA containing this specific code and bind to it, and there are other proteins which bind to the DNA-binding proteins. Together, these proteins form a complex structure that coats the chromosome ends and protects (or shelters) them from being mistakenly recognized as an accidental DNA break. Collectively, these proteins are therefore called the shelterin complex, and within this complex there are six different proteins: TRF1, TRF2, TIN2, TPP1, POT1, and hRAP1.

The repetitive DNA at the end of a chromosome is called a telomere. Sometimes, however, biologists use the same word to refer to the DNA plus the proteins that bind to it. Although this is potentially confusing, the meaning of the word is usually clear from the context.

Although telomeres still function despite a considerable amount of shortening, the amount of shortening that can occur without consequence is not unlimited. If too much shortening occurs, there is not enough telomere DNA left for shelterin to bind to, which results in the chromosome ends losing their protection from being treated as a DNA break.

Cells have a built-in mechanism to deal with shortened telomeres. Once a telomere reaches a minimum length, the cell is no longer permitted to divide again. In effect, it reaches its use-by date for replication. Telomere shortening can thus contribute substantially to the aging process. When there is a significant decline in the number of cells that are able to divide, tissues and organs lose their capacity to undergo the renewal processes upon which healthy function depends. Telomere shortening can contribute to aging even in tissues that contain many non-dividing cells (like the brain) because their health depends on cells that do need to continue dividing. For example, the nutrition of the non-dividing cells of the brain depends on blood vessels lined by cells that must continue dividing to maintain normal function.

How Telomeres Can Last a Normal Lifespan

Telomeres are part of a finely tuned biological system. Under normal circumstances, telomeres continue to function, protecting the chromosomes throughout all cell divisions of a normal human lifespan. Two critically important factors in their continued competence are their starting length and the rate at which they shorten.

Germline cells require processes to provide an adequate starting length very early in the development of the embryo. These processes involve the action of a complex molecular machine (enzyme) called telomerase, which is able to add new DNA (containing many repeats of the TTAGGG sequence) to the ends of chromosomes, and thereby completely counteract the normal shortening process that accompanies cell division. The similarity between the words "telomere" and "telomerase" is potentially confusing, so it is important to keep in mind that telomeres are the DNA at chromosome ends (which

become shorter with cell division), whereas telomerase is an enzyme (molecular machine) which lengthens telomeres.

The rate of telomere shortening is influenced by environmental and lifestyle factors. For example, toxic chemicals and cancer chemotherapy agents can cause tissue damage, and therefore induce a lot of cell division, thus increasing the overall rate of telomere shortening. Treatments that are normally used to get a patient ready for a bone marrow transplant can also cause this, and some viral infections can have this effect. In addition, lifestyle factors, such as physical inactivity, smoking, severe prolonged stress, and obesity are associated with shorter telomeres.

There are low levels of telomerase activity in the cells of many somatic tissues, which partially counteract normal telomere shortening. This slows down, but does not completely prevent the shortening in normal somatic cells. This is particularly important in organs that undergo a lot of cell division, including the bone marrow, which constantly produces huge numbers of new blood cells.

Telomerase is thus a key player in ensuring that our telomeres last for a normal human lifespan. It not only is important for providing a sufficient telomere length buffer at the start of life, but also for slowing down the rate of telomere loss through successive cell divisions. Inherited defects in telomerase can result in telomeres that are excessively shortened and therefore ineffective at preventing chromosome-related disease.

Telomerase

Telomerase is an enzyme that synthesizes the DNA sequence TTAGGG and adds it to the ends of telomeres. It does this by reverse transcribing an RNA molecule, which has a short sequence in the template region that is complementary to the TTAGGG DNA sequence. The RNA is sometimes referred to ashTER or hTERC, but usually hTR (human Telomerase RNA), and is encoded by the *TERC* gene (Telomerase RNA Component). When hTR molecules are being transcribed from the *TERC* gene, a string of additional

"letters" is added to its end. The letters are all "A", so this string is called the polyA tail. The level of hTR in a cell is finely tuned by enzymes that decrease (PARN) or increase (PAPD5) the length of the polyA tail. Increased length of the tail results in hTR being earmarked for destruction, and some drugs have recently been discovered which block the action of PAPD5 and therefore increase the level of hTR and of telomerase.

Telomerase also contains a protein subunit called TERT (Telomerase Reverse Transcriptase), which does the reverse transcribing. The name of the gene encoding this protein is *TERT*. A third component of the active telomerase complex is a protein called dyskerin, which binds to RNA molecules like hTR. The gene that encodes dyskerin is called *DKC1* because it was the first pathogenic variant confirmed to cause dyskeratosis congenita, one of the syndromes caused by short telomeres.

The active telomerase enzyme complex contains at least six molecules: two copies each of hTERT, hTR, and dyskerin. Assembly of this complex molecule requires the action of specialized proteins, including NOP10 and NHP2.

In order to lengthen telomeres, telomerase needs to be transported from the places where it is assembled to the ends of telomeres where it does its work. TCAB1 (encoded by the gene *WRAP53*) is a protein required for this transportation. TCAB1 (and also NOP10 and NHP2) may form part of the telomerase complex at various stages in its life cycle. Once telomerase arrives at the chromosome end, it needs to dock with the telomere. A protein critically important for this is TPP1 (encoded by the *ACD* gene). TPP1 has a small region on its outer surface (the "TEL patch") with which it latches onto the surface of the telomerase enzyme. TPP1 attaches to the telomere end by binding to another shelterin protein, POT1 (encoded by the *POT1* gene).

Other Molecules Needed for Normal Telomere Length

Considering data from other species, it would not be surprising to find that there are several hundred proteins that influence telomere length in humans to a greater or lesser extent.

Of those that are already known, the shelterin proteins are very important, because of their ability to influence telomerase activity and to protect the telomere. Telomerase synthesizes only the TTAGGG strand of telomere DNA. CTC1, STN1, and TEN1 are three proteins which form a molecular machine (the CST complex) which is thought to be involved in synthesizing the complementary CCCTAA DNA strand. The complex is also thought to be important in controlling the activity of telomerase.

The DNA of telomeres can be configured in a number of ways other than the helical Watson-Crick structure. The presence of many consecutive Gs in the telomere sequence means that telomeres are able to form complex structures (known as G-quadruplexes), whereby the Gs bind to each other instead of forming G-C pairs. Telomeres can also form a "t-loop" structure.

A number of proteins, including RTEL1, are required to prevent the G-quadruplex and t-loop structures from causing problems when telomeres are being copied during cellular replication (Figure 3).
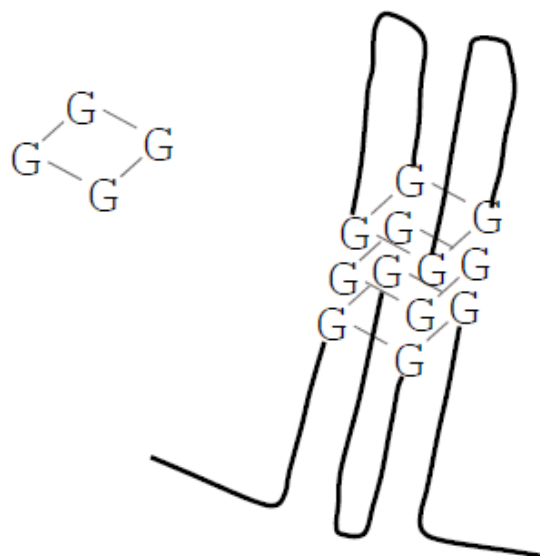
Figure 3. Telomeres can form structures called G-quadruplexes. DNA that contains many runs of the letter G, can fold into complex structures, where the Gs bond together (instead of binding to the Cs on the strand of complementary DNA). Four Gs can bond to form a square-like structure called a G-quartet (left), and stacks of G-quartets (right) are called G- quadruplexes.

Inherited Causes of Excessively Short Telomeres

Inherited defects (pathogenic variants) in any of the genes that encode components of telomerase (*DKC1*, *TERC*, or *TERT*) or of specific genes that encode proteins that control the level of hTR (*PARN*), hTR maturation (*ZCCHC8*), or are involved in telomerase's assembly (*NOP10*, *NHP2*, or *NAF1*), transportation to the telomere (TCAB1 encoded by *WRAP53*), or docking with the telomere end (TPP1, encoded by *ACD*), can result in telomeres being too short (Figure 4). In addition, defects in genes that encode specific proteins involved in other aspects of telomere protection and synthesis (*TINF2* [the gene encoding TIN2, one of the shelterin proteins], *CTC1*, *STN1*, *POT1*, and *RTEL1*) may also cause excessive telomere shortening. Some individuals who have excessively short telomeres do not appear to have variants in any of these genes, so it is likely that there

are additional inherited causes of short telomeres resulting in telomere biology disorders that have not yet been found.

Figure 4. Variant of a gene encoding one of the components of the molecular machinery responsible for maintaining telomere ends may result in a telomere biology disorder. The key components of telomerase are TERT, telomerase RNA (TR), and dyskerin. The amount of available TR is increased by PARN and ZCCHC8, and decreased by PAPD5. NOP10, GAR1, and NHP2 help to assemble TERT, TR, and dyskerin to form telomerase, which is transported ("trafficked") to the telomere by proteins that include TCAB1, where it docks with TPP1 and lengthens the TTAGGG strand of the telomere. The CST complex (which includes CTC1) is required for synthesis of the CCCTAA strand. The protection and normal function of the telomere also depends on RTEL1 (which unwinds G-quadruplex structures to allow the telomere to be copied when a cell is undergoing replication), and of a large conglomerate or proteins which are collectively called shelterin. The components of shelterin include TPP1 and TIN2.

The consequences of deficient telomerase activity are two-fold (Figure 5).

First, the normal shortening that accompanies cell division is counteracted less effectively than when telomerase is normal, so telomeres shorten faster. Second, when there is less telomerase activity than normal, it may not be possible to restore telomere length in the germline cells and early embryo. The result is that an individual in the next generation inheriting the defective gene may also inherit telomeres that start off shorter than normal, a "double whammy" effect. This results in a tendency for some diseases with short telomeres to get worse from generation to generation, an unusual pattern of inheritance called genetic anticipation.

Defects in the other genes which cause short telomeres may follow a similar pattern, even though they may not directly affect telomerase activity. Gene defects causing failure to protect the telomere or to copy the telomere DNA properly during cell division may speed up the rate of telomere shortening to an extent that normal levels of telomerase are not able to adequately counteract, either in somatic or germline cells that pass DNA on to the next generation.
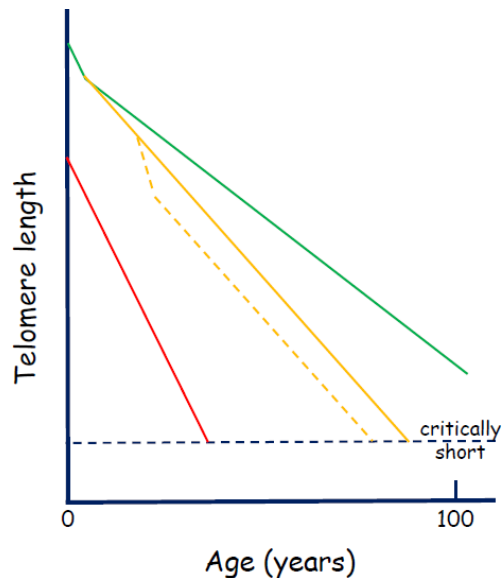
Figure 5. Telomeres need to last for a normal life span. The telomeres of many somatic cells shorten throughout life but, if they have a normal starting length and do not shorten too rapidly, we can live a long life and still have sufficient cells that do not have critically short telomeres (green line). Because of the amount of growth occurring, telomeres shorten more rapidly in early childhood than in adults. If the telomeres shorten faster than normal (which occurs, for example, when cells that undergo a lot of proliferation do not have enough telomerase), then some organs may lose the capacity to renew themselves later in life (amber line). Some environmental factors may cause cell death, which results in an increased need for proliferation and therefore, an increased rate of telomere shortening, and this may result in problems occurring earlier in life (dotted amber line). Individuals who start life with short telomeres and also have an increased rate of telomere shortening (red line) may have problems from short telomeres early in life.

Why Short Telomeres Cause Disease

Cells with excessively short telomeres reach their proliferative "use-by date" much earlier than normal, which means that various tissues and organs are not able to maintain themselves by normal numbers of cell divisions. Eventually, this may result in an insufficient number of cells (cytopenia) in various organs.

The severity of the condition tends to be related to how short the telomeres are. If the telomere length deficit is very severe, there may even be insufficient cell division for organs to develop normally in specific embryonic tissues (for example, in the cerebellum and other parts of the brain). When telomere biology disorders become manifest first in childhood, they often affect the bone marrow, which is normally one of the most highly proliferative organs. This causes a deficiency in numbers of red blood cells (anemia), white cells (neutropenia), and platelets (thrombocytopenia). The combination of these three deficiencies is called aplastic anemia or bone marrow failure. When telomere shortening is less severe, problems may not surface until later in life.

Excessively short telomeres are also associated with an increased risk of cancer. The reasons for this may include the following. First, when telomeres become excessively short, they lose their ability to protect chromosome ends from the DNA repair machinery. This results in end-to-end fusion of chromosomes and the potential for the joined chromosomes to break at a random location when the cell next divides. This sets up a cycle where continued random chromosome breakage and random rejoining creates unpredictable changes in the genome, which may increase the risk of cancer. Second, organs that are depleted of normal cells may send increasingly powerful signals to stimulate the remaining cells to divide, which may inadvertently favor the growth of rogue cells on their way to becoming cancerous. Third, normal cells in a normal organ can often restrain the growth of rogue cells, but this effect is progressively lost as surrounding cells die.

Excessive telomere shortening can affect almost any organ system, but it is still not clear why it causes bigger problems in some organs than others. It seems easy to understand why bone marrow, a highly proliferative tissue, may be affected. It is difficult to understand, however, why there are more often serious problems in lungs, which are thought to have only moderate rates of cell division, than in the skin, or in the lining of the gastrointestinal tract, both of which constantly undergo high levels of cell division. It is also difficult to understand, for example, why some individuals who have no major

problems with their bone marrow will develop lung disease. Even more baffling, there are individuals with very short telomeres who appear to be disease-free for a normal life span.

The answers may lie in part in the effects of environmental and lifestyle factors. In some families with short telomeres, pulmonary disease only occurs in individuals who have both inherited the defective gene and who smoke. In other families, an individual's short telomere-related problems may become manifest when treated with chemotherapy for cancer. The answers may also lie in the modifying effects of other genes we do not yet know about.

This uncertainty also provides some grounds for optimism. An individual who inherits a pathogenic variant associated with short telomeres may not necessarily develop any manifestations of the condition, and even if one or more of these manifestations occur, disease progress may be quite unpredictable. The more that is understood about interactions between the environment and telomere biology, the better we may be able to prevent or modulate the adverse effects of the genes.

Chapter 3

Diagnosing Telomere Biology Disorders

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Introduction

Dyskeratosis congenita (DC) was the first Telomere Biology Disorder (TBD) recognized in the biomedical literature and initially defined by the mucocutaneous triad of lacy reticulated skin pigmentation, nail dystrophy and oral leukoplakia (see Chapter 1, Introduction) [1]. In most cases, DC presents over the first two decades of life. In others, symptoms begin to appear in early adulthood [2, 3]. DC is related to three other syndromes that present in infancy or early childhood, Hoyeraal Hreidarsson syndrome (HH), Revesz syndrome (RS), and Coats plus, which, like DC, arise from defective telomeres (see Chapter 2, Why Telomeres Matter). Further expanding what is now appreciated as a spectrum of TBDs is telomere-mediated pulmonary fibrosis, which typically presents in later adulthood. This chapter will discuss the importance of telomere length testing in rendering a diagnosis of a TBD, describe the evaluation and clinical features of the various TBDs, basic diagnostic criteria, and specific genetic associations. While the specific diagnostic

labels do convey important clinical information, there is clinical overlap and, importantly, clinical features can evolve over time or may be apparent only with systematic evaluation.

Telomere Length Testing

Several methods have been developed to measure telomere length:

- **Automated multicolor flow cytometry combined with fluorescent *in situ* hybridization (flow FISH).** Flow FISH provides a measurement of average telomere length in cells of leukocyte subsets [4, 5]. It is the only test that is clinically available in certified labs and validated for the diagnosis of TBDs.
- **Southern blot analysis of telomere restriction fragments.** This method is often used in biomedical research and aided the initial discovery of DC as a TBD. Given its various limitations, it is not suitable nor available for clinical diagnostic purposes.
- **Telomere quantitative polymerase chain reaction (qPCR)** [6, 7]. While the stalwart of epidemiologic telomere-related research, qPCR is less accurate, reproducible, sensitive and specific for the diagnosis of TBDs than flow FISH [8].
- **High throughput single telomere length analysis (HT-STELA).** This newer approach, which can measure very short telomeres, may emerge as an additional sensitive and specific clinical test for TBDs with further development and research [9].

Flow FISH as a diagnostic test: Clinically certified testing of telomere length by flow FISH is available in the USA, Canada, Switzerland, Germany, and Australia (see

Resources). Importantly, since this testing is performed on fresh peripheral blood cells, it may only be used as a diagnostic tool prior to hematopoietic cell transplantation (HCT); after HCT, donor, rather than native, cells would be assayed. Individuals with DC, HH, and RS have very short telomere lengths across cell types, defined as telomere lengths less than the first percentile for age [10-13]. Specifically, very short telomere length in practically all leukocyte subsets (granulocytes, naïve T cells, memory T cells, B cells, and NK/NKT cells) as determined by flow FISH is both highly sensitive and specific for a diagnosis of one of these TBDs (see Table 1 and Figure 1) [10-12]. The severity of disease correlates with telomere length with the most severely affected, typically those with RS or HH, having the greatest degree of telomere shortening from the age-adjusted mean [12].

Table 1. Telomere lengths in DC patients compared with DC relatives [12].

	DC Patients N abnormal	DC Relatives N abnormal	OR	95% CI	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Granulocytes	60/62	22/123	138	31-1200	97	82	73	98
Lymphocytes	63/65	11/127	332	68-2942	97	91	85	98
CD45RA$^+$/CD20$^-$ naïve T cells	61/64	9/127	266	64-1468	95	93	87	98
CD45$^-$ memory T cells	61/64	11/127	214	53-1161	95	91	85	98
CD20$^+$ B cells	54/58	12/127	129	37-546	93	91	82	97
CD57$^+$ NK/NKT cells	50/59	12/119	50	18-140	85	90	81	92
≥4/6 lineages	61/64	9/117	244	58-1346	95	92	87	97
≥3/5 lymphocyte lineages	62/64	9/119	379	74-3390	97	92	87	98

4/4 lymphocyte subsets	42/55	7/127	55	19-170	76	94	86	90
≥3/4 lymphocyte subsets	54/55	7/127	926	113-37479	98	94	89	99
3/3 naïve and memory T and B cells	51/58	7/127	125	38-437	88	94	88	94
≥2/3 naïve and memory T and B cells	57/58	9/127	747	97-30241	98	93	86	99
2/2 naïve and memory T cells	59/64	7/127	202	55-806	92	94	89	96
1/2 naïve and memory T cells	63/64	13/127	552	77-22333	98	89	83	99
Granulocytes + lymphocytes	58/65	11/127	87	30-273	89	91	84	94

Denominators vary according to the number of patients in whom each included lineage had sufficient numbers of cells for analysis. The best performance characteristics are in lymphocytes alone, and at least three of the four lymphocyte subsets. Abnormal: below the first percentile for age in normals. OR: odds ratio in favor of being a DC patient compared with an unaffected relative. CI: confidence interval; sens: sensitivity; spec: specificity; PPV: positive predictive value; NPV: negative predictive value. Table reproduced from Alter et al., *Haematologica* 2012 [12]. Note, the DC population (n=65) included those with classical DC (40), HH (14), RS (4) or were silent carriers (7).
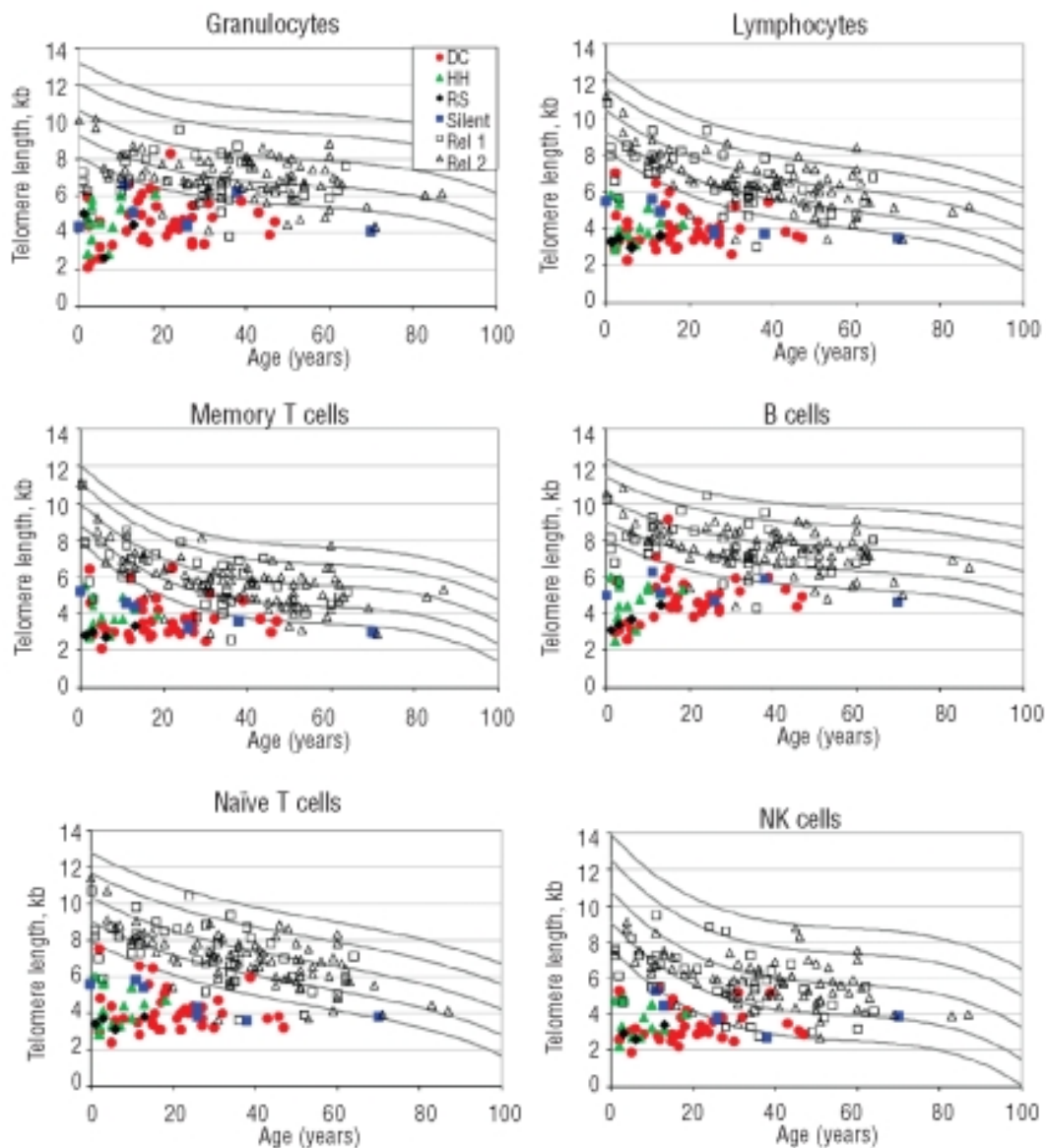
Figure 1. Telomere length according to age in patients with DC and related TBDs and their relatives. The vertical axis represents telomere length in kb. The curved lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal controls. Colored symbols represent patients with DC and their relatives. Red circles: classical DC patients; green triangles: HH; black diamonds: RS; blue squares: silent carriers; open black squares: DC relatives in families with unknown genes; open black triangles: DC relatives without pathogenic variants in the probands' genes. Top panels show granulocytes, lymphocytes, and CD45RA+/CD20- naïve T cells. Bottom panels show CD45RA- memory T cells, CD20+ B cells, and total NK/NKT cells. Figure adapted and Figure Legend directly from Alter et al., *Haematologica* 2012 [12].

Telomere length slightly below the first percentile in three of the lymphocyte populations is only very rarely observed in patients with other inherited bone marrow failure syndromes, such as Fanconi anemia, Diamond-Blackfan anemia, Shwachman-Diamond syndrome [10-12]. Very short lymphocyte telomere lengths may also be found in association with hepatitis-associated severe aplastic anemia, with lengths increasing into normal range following treatment [14]. Additionally, individual patients with several rare disorders, such as *LIG4*-, *CORO1A*-, and *RUNX1*-associated disease, have been reported to have very short telomere lengths [15-17]. Further study is needed in these rare disorders to better understand the significance of these findings. Thus, while highly sensitive and specific for DC/RS/HH, very short telomeres alone are insufficient to render a TBD diagnosis. Results should be interpreted in the context of the patient's other clinical features and family history.

Bone marrow failure can precede the development of other DC features. Therefore, telomere length in leukocyte subsets by flow FISH testing is highly recommended for all patients with aplastic anemia. This is particularly true for patients being considered for HCT, as the diagnosis of a TBD has a major impact on the conditioning regimen and may influence donor selection. For example, siblings with very short telomeres would be considered suboptimal donors even in the absence of overt disease (see Chapter 13, Hematopoietic Stem Cell Transplantation). Additionally, medical treatment for TBD-related bone marrow failure might include androgens, while immunosuppressive therapy might be indicated in cases of acquired bone marrow failure (Chapter 10). Thus, telomere length testing has the potential to greatly influence treatment strategy.

In contrast to DC, HH, and RS, the extent of telomere shortening observed in individuals with Coats plus when measured by flow FISH varies from well below to just at the first percentile [18]. Analysis by qPCR and telomere restriction fragment analyses have also yielded telomere lengths in normal range [19, 20]. Thus, flow FISH results may not aid in the diagnosis of Coats plus. Laboratory-based research studies have nonetheless uncovered telomere defects associated with Coats plus variants, consistent with the role of genes mutated in Coats plus, *CTC1*, *STN1* and *POT1*, in telomere biology [20-23].

Lastly, adult-onset TBDs, such as telomere-mediated pulmonary fibrosis, have telomeres that may be below or within the first to tenth age-adjusted percentiles by flow FISH [24].

Dyskeratosis Congenita

Classic DC is diagnosed by the presence of the mucocutaneous triad. DC, however, can impact every organ system and lead to a wide range of clinical manifestations, with bone marrow failure being a major clinical feature (see Table 2) [25, 26]. The identification of pathogenic germline genetic variants causative of DC and development of telomere length testing have facilitated diagnosis (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families). As a result, diagnostic criteria for DC have evolved over the past two decades, although expert opinions vary in the details [26-29]. The term DC is now widely used to also describe patients presenting during the first two decades of life with very short telomeres, with or without a pathogenic variant in a known TBD gene, and bone marrow failure or multiple other clinical features as outlined in Table 2.

Major Clinical Features of DC

1. **The mucocutaneous triad**

   The mucocutaneous triad of reticulated skin pigmentation, nail dystrophy, and oral leukoplakia typically manifests in mid-to-late childhood. The features need not present simultaneously nor do all need to be present to make a diagnosis of DC (see Chapter 6, Dermatologic Manifestations).

   a. Reticulated skin pigmentation (Figure 2)

   Skin changes most often appear as reticular or lacy hypo- and hyperpigmentation, but may also be more punctate. All areas of skin may be affected, although changes may be restricted to neck, upper chest, and proximal parts of the limbs initially. In some cases, the pigmentation follows Blaschko lines [30]. Skin findings may simulate manifestations of graft versus host disease, a complication of HCT. Some unrelated disorders also manifest reticular skin pigmentation including dermatopathia pigmentosa reticularis, Naegeli syndrome, poikiloderma with neutropenia (also known as poikiloderma Clericuzio type), and Kindler syndrome.

Figure 2. Skin pigmentation changes in TBDs. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

b. Nail dystrophy (Figure 3)

Changes to the finger and toe nails may be subtle or severe, with ridging, thinning, peeling, or slow growth. Nail changes in a given patient may be asynchronous, with normal appearing nails adjacent to nails that are clearly affected. With age, nails may even seem to "disappear".

Figure 3. Nail dystrophy in TBDs. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

c. Oral leukoplakia (Figure 4)

Oral leukoplakia appears as thickened, white patches that cannot be scraped off the buccal mucosa or along the edges and surface of the tongue. An experienced otolaryngologist (ear, nose, and throat doctor) or oral surgeon best evaluates oral leukoplakia.

Figure 4. Leukoplakia in TBDs Image 1 and 2 from Savage and Bertuch *Genet Med*. 2010 [28]. Image 3 obtained after informed consent from participant in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

2. **Bone marrow failure**

Bone marrow failure is generally defined as bone marrow cellularity less than normal for age, and with one or more peripheral blood cytopenias [absolute neutrophil count, hemoglobin (reflecting red blood cell count), or platelet count below the lower limit of normal for age]. It is a common feature of DC, with up to 85% of patients in the London Dyskeratosis Congenita Registry reporting bone marrow failure by the age of 30 years [25]. In a competing risk analysis, the cumulative incidence of bone marrow failure in the National Cancer Institute Inherited Bone Marrow Failure Syndrome Study was 50% by age 50 [31]. The extent of bone marrow failure can be mild to severe, and can precede the mucocutaneous features of DC. Bone marrow failure at any age should prompt consideration of a diagnosis of DC or related TBD.

Diagnostic evaluations for bone marrow failure include:
- Complete blood count, including mean corpuscular volume (MCV). An elevated MCV may indicate long standing stress erythropoiesis, as occurs in DC and other inherited bone marrow failure syndromes, rather than more acute bone marrow failure as in most cases of immune aplastic anemia.
- Absolute reticulocyte count
- Hemoglobin F measurement. As with MCV, an elevated hemoglobin F measurement may reflect an underlying inherited bone marrow failure syndrome rather than an acute marrow failure process.
- Bone marrow aspiration and biopsy
- Bone marrow cytogenetic analysis by G banding
- Bone marrow fluorescence in situ hybridization to detect 5q-, 7q-/monosomy 7, trisomy 8 and 20q-, if clinically indicated.

Additional evaluations that may be considered:

- In the absence of nail dystrophy or reticulated skin pigmentation, chromosome breakage analysis should be performed to rule out Fanconi anemia. Because individuals with Fanconi anemia may also develop leukoplakia, this finding cannot be used to distinguish DC from Fanconi anemia.
- RBC folate and vitamin B12 to assess stores if MCV is elevated.

Testing available but of uncertain utility:

- Next-generation sequencing (NGS) panels to detect somatic variants in blood or bone marrow samples in genes associated with sporadic hematologic malignancies and aplastic anemia are now readily available. However, prognostic utility of identifying a variant in such a gene in patients with a TBD is not known.

The hematologic manifestations of DC and related TBDs and their treatment are presented in detail in Chapter 10, Medical Management of Bone Marrow Failure in Telomere Biology Disorders.

Identifying Additional Features of DC and Related TBDs

Table 1 lists the multitude of clinical findings that may be observed in DC. Some of these findings may be apparent on physical examination, whereas others require specific testing. Clinical evaluations that may be done to uncover additional features of DC and related TBDs are listed below. These should be considered on an individual patient basis, as clinically indicated.

Table 2. Diagnostic Findings in DC. These features present with variable severity and may not be present in all individuals

Physical Features	
Mucocutaneous triad	Dystrophic nails
	Lacy reticulated pigmentation, especially neck and thorax
	Leukoplakia (white patches), usually oral
Additional features (in order of frequency) [26]	
Eyes	Epiphora (tearing), lacrimal duct stenosis, blepharitis, exudative retinopathy
Hair	Early graying, loss, sparse eyelashes
Gastrointestinal	Esophageal stricture; liver fibrosis, cirrhosis; hepatopulmonary syndrome; peptic ulceration, enteropathy
Stature	Short
Dental	Caries, missing teeth, periodontitis, decreased crown/root ratio, taurodontism
Skeletal	Osteoporosis, hip avascular necrosis
Head/ Neuro-developmental	Microcephaly, cerebellar hypoplasia (ataxia, spasticity, hypotonia), intracranial calcification
Perinatal	Low birth weight, intrauterine growth restriction
Lung	Fibrosis, restrictive; arterio-venous malformations

Males	Small testes, undescended testes; phimosis, meatal stenosis, urethral stricture, hypospadias, leukoplakia
Skin	Hyperhidrosis

Neurodevelopmental
Learning disability, developmental delay, intellectual disability, depression, anxiety

Laboratory Features	
Blood	Anemia, and/or thrombocytopenia, and/or neutropenia
	Pancytopenia
	High MCV for age
	High fetal hemoglobin (Hb F) for age
Bone Marrow	Aplastic: Hypocellular for age
	Myelodysplastic syndrome: significant dyspoieses (per WHO classification) +/- cytogenetic clone
	Leukemia: > 20% blasts in marrow
Telomeres	Below first percentile for age by automated multicolor flow-FISH in three of four lymphocyte subsets (CD45+ naïve T cells, CD45- memory T cells, CD20+ B cells, CD57+ NK/NKT cells) and granulocytes
Genes	Pathogenic variant in a DC-associated gene

Growth delay

- Birth weight and length measurements and gestational age at birth to assess for intrauterine growth restriction
- Current weight and length to assess for short stature and/or failure to thrive

Developmental delay/intellectual disability (see also Chapter 24, Neuropsychiatric Complications)

- Neuropsychological testing

Ophthalmologic manifestations (see also Chapter 7, Ophthalmic Manifestations) [32]

Examination should be performed by an ophthalmologist and include a retinal exam. Findings may include:

- Epiphora (constant tearing) due to lacrimal duct stenosis or its congenital absence
- Blepharitis
- Retinal neovascularization
- Retinal hemorrhages
- Exudative retinopathy, can be observed in some patients with DC but should prompt consideration of RS or Coats plus

Hearing loss

- Audiogram or auditory brain-stem evoked response testing

Dental involvement (see also Chapter 8, Dental and Oral Complications)

In addition to oral leukoplakia, screening should allow for detection of:

- Extensive caries or tooth loss
- Periodontal disease

- Taurodontism (enlarged tooth pulp chambers) or decreased tooth root/crown ratio

Lung involvement (see also Chapter 14, Pulmonary Fibrosis and Chapter 15, Lung Transplantation)

Initial evaluations to assess involvement of the lungs include:

- Pulse oximetry
- Pulmonary function tests (PFTs)
- Diffusion capacity of the lung for carbon monoxide (DLCO testing)
- Six-minute walk test for young children unable to perform PFTs

In cases in which lung involvement is suspected, additional testing includes:

- Chest radiography
- Non-contrast high resolution chest computed tomography
- Agitated saline echocardiogram or bubble study

Gastrointestinal tract and liver involvement (see also Chapter 17, Gastrointestinal Disease - Luminal, Chapter 18, Hepatic Complications, and Chapter 19, Liver Transplantation)

- A patient may report dysphagia due to the presence of an esophageal web or stricture, which is diagnosed by barium swallow or esophagram.
- Upper and lower gastrointestinal tract bleeding due to ulceration, telangiectasias, or varices may be diagnosed by upper and lower tract endoscopy.
- Liver disease may be revealed by the following testing:
    - Aspartate aminotransferase (AST/SGOT)
    - Alanine aminotransferase (ALT/SGPT)
    - Alkaline phosphatase (Alk phos)
    - Gamma-glutamyltransferase (GGT)
    - Conjugated and unconjugated bilirubin

- ○ Albumin
  - ○ Prothrombin time (PT)
  - ○ Ammonia
  - ○ Liver ultrasound with Doppler, liver elastrography (fibroscan), or MRI
  - ○ Liver biopsy, which may be indicated if the above studies are abnormal, should include assessment of liver iron stores in addition to histopathology

Genitourinary tract involvement (see also Chapter 20, Genitourinary Complications)

- Physical examination may reveal
  - ○ Urethral stricture
  - ○ Hymenal stricture in females
  - ○ Phimosis in males
  - ○ Hypogonadism (small testes) in males
- Urinalysis may uncover microscopic hematuria due to hemorrhagic cystitis or ureteral bleeding.

Musculoskeletal and endocrine disease (see also Chapter 22, Endocrine and Skeletal Disorders)

Complaints of hip or shoulder pain may be due to avascular necrosis (AVN) of the humeral or femoral head. AVN can be diagnosed by:

- X-ray – most sensitive for late-stage disease
- Bone scan
- MRI – may pick up early changes in bone

Osteoporosis may be present and is diagnosed by:

- Dexascan
- Spine X-ray, which may also reveal compression fractures

Additional mucocutaneous findings (see also Chapter 6, Dermatologic Manifestations)

- Atrophy of the papillae on the dorsum of the tongue
- Complete or patchy alopecia
- Premature graying of the hair
- Sparse eyebrows and lashes
- Telangiectasias
- Hyperpigmentation of the gums, tongue, palms, and soles have been anecdotally reported in individuals of African descent
- Glyphs (fingerprints) may disappear over time

Immunologic abnormalities (see also Chapter 11, Immunologic Complications in Dyskeratosis Congenita and Hoyeraal-Hreidarsson Syndrome)

Patients may present with common variable immunodeficiency [33, 34]. Severe combined immunodeficiency, if present, warrants further consideration of HH as discussed below. Testing for immunodeficiency may include:

- Determination of T, B, and NK cell percentages and absolute numbers
- Quantitative immunoglobulin levels for IgG, IgM, IgA, and IgE
- Lymphocyte proliferation panel for mitogens and antigens

Neurologic manifestations

- Frontal-occipital head circumference measurement to detect microcephaly
- Brain magnetic resonance imaging (MRI) to detect cerebellar hypoplasia (Figure 4), cerebral atrophy, corpus callosum abnormalities, small pons [35]
- Head X-ray or brain computed tomography (CT) to detect calcification
- Brain MRI or CT may also reveal cerebral cysts
- Neurodevelopmental assessment

Figure 5. Cerebellar hypoplasia in Hoyeraal-Hreidarsson syndrome [35].
Abbreviations: mo, months old; yo, years old; HH, Hoyeraal Hreidarsson syndrome.
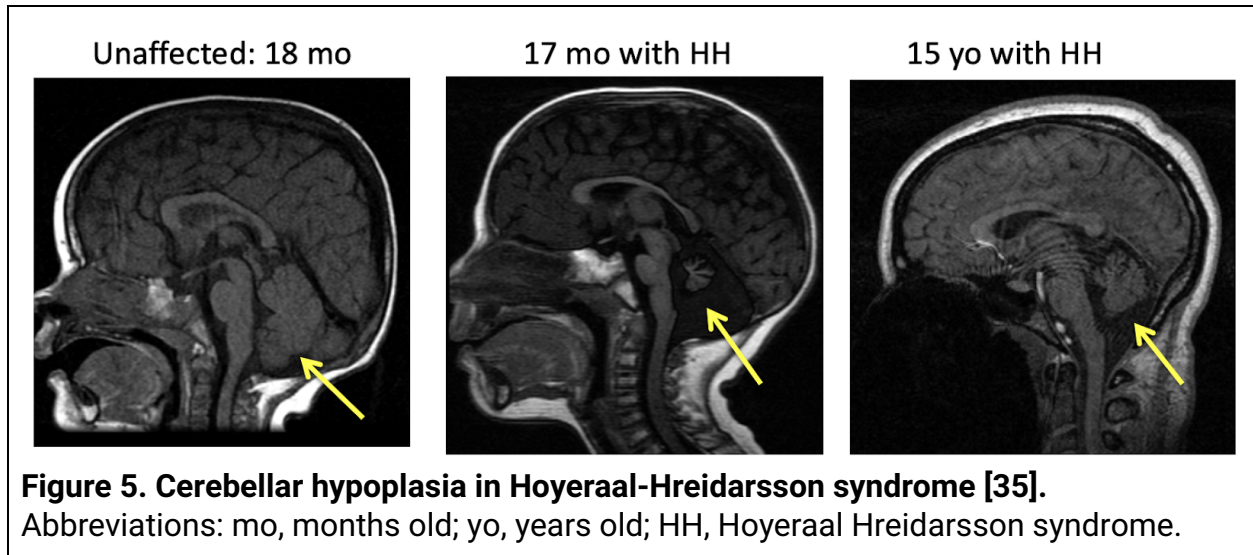
Pertinent History

Cancer history of patient (see also Chapter 9, Solid Tumors)

Cancer may be the presenting feature of DC. The most common neoplasms seen in DC include:

- Myelodysplastic syndrome and acute myeloid leukemia
- Head/neck cancer, especially squamous cell carcinoma of the tongue
- Anogenital squamous cell carcinoma

Family History

Obtaining a thorough family history is crucial. As discussed in Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families, disease anticipation, with earlier onset and more severe disease manifestations, may be observed in successive generations. Patient pedigree may include history of:

- DC, HH, RS, Coats plus
- Pulmonary fibrosis
- Liver fibrosis or cirrhosis of nonalcoholic, noninfectious etiology

- Hepatopulmonary syndrome
- Bone marrow failure, myelodysplastic syndrome, leukemia or lymphoma
- Cancer in young relatives (age less than 50 years), especially of the head and neck
- Infant or early childhood death due to immunodeficiency or severe enterocolitis

Molecular Diagnosis

Gene sequencing

To date, pathogenic variants in 15 genes have been found to cause TBDs: *ACD*, *CTC1*, *DKC1*, *NAF1*, *NOP10*, *NHP2*, *PARN*, *POT1*, *RTEL1*, *STN1*, *TERC*, *TERT*, *TINF2*, *WRAP53* (TCAB1), and *ZCCHC8* [36-39]. (See Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families for further discussion of the genetics of the TBDs.) *MDM4* has been recently proposed as an additional TBD gene but awaits additional evidence [40]. Clinical laboratories offer NGS panels of many of these genes, and whole exome sequencing is also available as an alternative approach. Copy number analysis via chromosomal microarray or NGS may also uncover pathogenic changes in TBD genes. Sequencing of the known TBD-associated disease in large cohorts of individuals, including both children and adults, with a clinical diagnosis of a TBD will identify causative variants in ~70-80% of cases. It is important to recognize that the absence of a known disease-associated variant does not rule out a TBD. In addition to aiding the diagnosis of an individual patient, obtaining a molecular diagnosis through gene sequencing provides a mechanism to screen family members and to offer genetic counseling, as well as permit pre-implantation genetic diagnosis (see Chapter 5, Genetic Counseling for Families).

Additional Considerations

Female carriers of *DKC1* pathogenic variants

Although *DKC1* variants result in X-linked recessive disease [41], female carriers may occasionally manifest clinical features of a TBD, such as delayed wound healing, abnormal pigmentation, and nail dystrophy [42]. In addition, they may have skewed leukocyte X chromosome inactivation [30] defined as greater than 90 percent of cells inactivating the same X chromosome (for example, the X chromosome inherited from the mother). Clinical X chromosome inactivation analysis utilizing the human androgen receptor assay is offered by numerous testing facilities, and may be useful in determining the carrier status of females when *DKC1* testing is not available or a variant of uncertain significance is identified.

Revertant somatic mosaicism

Similar to that seen in Fanconi anemia, revertant somatic mosaicism has been reported in autosomal dominant DC [43]. This phenomenon refers to the presence (within the same person) of cells bearing a variant originating from the germline, as well as a subpopulation of cells in which the abnormal (mutant) allele has reverted to wildtype. Reversion is thought to occur via mitotic homologous recombination. These hematopoietic stem cells no longer bear a DC-associated gene variant, and so may have stabilized or possibly lengthened the telomeres. They may thereby have the potential to drive effective hematopoiesis. Such growth advantage is not observed in other somatic tissues like lung, liver, and skin. These patients may have clinical features suggestive of DC but with minimal hematopoietic abnormalities. Sequencing of blood cell DNA may fail to detect the presence of the mutant allele due to its relatively smaller proportion in peripheral blood. Therefore, in cases in which revertant somatic mosaicism is suspected, for example in patients with solely extrahematopoietic manifestations of DC, DNA from nonhematopoietic tissue, like skin fibroblasts, should be analyzed.

Hoyeraal-Hreidarsson Syndrome

A diagnosis of Hoyeraal-Hreidarsson syndrome (HH) should be considered in children meeting diagnostic criteria of DC (see above), plus

- Cerebellar hypoplasia

Additional features with high penetrance in HH include

- Intrauterine growth restriction
- Developmental delay and intellectual disability
- Microcephaly
- Immunodeficiency

Classic HH typically presents in early childhood as a progressive, multisystem disorder. Cerebellar hypoplasia is considered by most as a defining feature of HH and may result in signs of cerebellar dysfunction such as ataxia and speech difficulties. Additional central nervous system findings include delayed myelination, hydrocephalus, brain atrophy, and calcification [44]. Microcephaly is frequently present.

Immunodeficiency is an additional major feature and may progress to severe combined immunodeficiency syndrome (SCID) of the T+, B-, NK- cell type, with lethal viral infection in infancy [45-47]. This has raised the possibility that there are young patients with SCID who succumb to infection prior to the recognition of underlying HH. Gastrointestinal problems with chronic bloody diarrhea and feeding difficulties have also been reported [48]. The mucocutaneous triad and additional features of DC may also be present [49].

Historically, the vast majority of individuals with HH reported in the literature have died within the first decade of life due to immunodeficiency or bone marrow failure [50]. However, with improved diagnosis, supportive care, and HCT, longer term survival is possible today [51, 52].

All of the genes associated with HH to date are associated with telomere maintenance (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders). These are:

- *DKC1*, which transmits X-linked recessive HH [53], accounting for the large male preponderance
- *TINF2*, which results in sporadic HH due to de novo heterozygous pathogenic variants
- *ACD*, *TERT*, and *RTEL1*, which result in autosomal recessive HH due to either compound heterozygous or homozygous pathogenic variants

The carrier frequency of the HH-associated *RTEL1* variant c.3791G>A (p.R1264H) is 1% in the orthodox Ashkenazi Jewish and 0.45% in the general Ashkenazi Jewish populations [54]. Therefore, targeted sequencing may be considered initially in these populations.

In addition to variants in the above genes, a heterozygous splice variant of *DCLRE1B* (SMN1B), which encodes the nuclease Apollo, was reported in a child with HH [55]. Apollo is implicated in telomere maintenance, the hallmark abnormality of HH, but it also has a role in certain forms of general DNA repair. In contrast to most individuals with HH who have very short telomeres, the case with the *DCLRE1B* splice variant lacks a defect in telomere length [55], although there is evidence of telomere dysfunction [47]. Thus, telomere length above the first percentile does not necessarily rule out a diagnosis of HH.

Revesz Syndrome

A diagnosis of Revesz syndrome (RS) should be considered in a child who meets diagnostic criteria of DC (see above) plus

- Bilateral exudative retinopathy (bilateral Coats disease)

Additional features may include

- Intrauterine growth restriction
- Sparse hair
- Intracranial calcification

RS is another pediatric onset TBD, with the defining feature of bilateral exudative retinopathy, also known as Coats disease. (See Chapter 7, Ophthalmic Manifestations for further information on ophthalmologic manifestations of TBDs, including RS.) Additional features of RS include intrauterine growth restriction, intracranial calcification, sparse hair, and bone marrow failure [56]. Patients may also have microcephaly, cerebellar hypoplasia, failure to thrive, and additional features of DC, including components of the mucocutaneous triad, most often nail dystrophy and least often oral leukoplakia [57].

The phenotypic overlap of RS and DC has long been appreciated [58]; however, only 18 cases of RS have been well described in the medical literature (clinical features of each tabulated by Karremann, et al) [57]. Of those reported, the vast majority present to medical attention before the age of five years, with the original case described in a six month old infant [56]. Bone marrow failure is most often evident by the second year of life. This early age of presentation, along with the severity and spectrum of disease manifestations, has led to the frequent description of RS as a severe variant of DC. Consistent with this, patients with RS not only have very short telomeres, but telomeres that are shorter than in patients with classic DC and similar to those observed in HH [13]. Lastly, a slight majority of reported cases are males; whether this reflects a true male predilection or simply represents a reporting or recognition bias remains unknown.

The only gene found to date with pathogenic variants in patients with RS is *TINF2*, which encodes TIN2, a member of the telomeric shelterin complex (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders) [59, 60]. Therefore, targeted sequencing of *TINF2* is a reasonable first step toward a molecular diagnosis in a patient with RS. Not all patients with RS, however, will have a *TINF2* pathogenic variant

[12]. Heterozygous *TINF2* pathogenic variants are also associated with classic DC and HH, and are often de novo [60]. While it is probable that most cases of *TINF2*-associated RS are due to *de novo* pathogenic variants, there is one case in the literature of RS in which the *TINF2* variant was inherited, although the carrier parent was a mosaic [61].

A family has been described in which two siblings with exudative retinopathy were found to carry a novel *TERT* variant, c.2603A>G, p.D868G [62]. Although these children had very short telomeres, bone marrow failure, and early pulmonary fibrosis, as seen in DC, they did not have the intracranial calcifications or neurodevelopmental deficits frequently observed in RS. A large number of *TERT* variants have been reported in the literature, including homozygous pathogenic variants in patients with very short telomeres. These are not reported to be associated with exudative retinopathy, so it remains to be determined whether the ocular phenotype in this family is due to the *TERT* variant or is unrelated.

Coats Plus

The diagnosis of Coats plus is made by the presence of the following clinical features:

- Distinctive pattern of intracranial calcification involving the thalamus, basal ganglia, dentate, and deep cortex, with associated leukoencephalopathy and brain cysts
- Retinal telangiectasia and exudates (as seen in Coats disease)
- Osteopenia with tendency to fracture and with poor bone healing
- Recurrent gastrointestinal hemorrhage due to vascular ectasias in the stomach, small intestine, and liver
- Intrauterine growth restriction
- Additional features overlapping with DC may be present: dystrophic nails, sparse hair, and abnormal skin pigmentation

Coats plus is the clinical syndrome most recently placed within the spectrum of TBDs. Similar to RS, patients with Coats plus have bilateral exudative retinopathy or telangiectasias, as well as a characteristic pattern of asymmetric intracranial calcification involving the thalamus, basal ganglia, dentate, and deep cortex, with associated leukoencephalopathy and brain cysts; osteopenia with tendency to fracture and poor bone healing; recurrent gastrointestinal hemorrhage due to vascular ectasias in the stomach, small intestines and liver; and pre- and postnatal growth restriction [63]. Additional features include the mucocutaneous triad of DC and bone marrow involvement, although not typically marrow failure.

Consistent with these overlapping clinical features of DC, the vast majority of patients with Coats plus have biallelic variants in *CTC1*, a gene that encodes a factor important for telomere maintenance (see Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders) [18, 19] and patients diagnosed with classic DC have also been found to have biallelic *CTC1* pathogenic variants [64, 65]. In addition to *CTC1*, biallelic pathogenic *STN1* variants have been reported in three unrelated patients with Coats plus [20, 66], an expected association given that *CTC1* and *STN1* proteins form a complex. Lastly, a homozygous pathogenic *POT1* variant was identified in two siblings with Coats plus, implicating this shelterin complex gene in Coats plus as well [23].

Whether very short telomeres are a molecular feature of *CTC1*-associated disease remains to be determined. In the initial two reports on patients with Coats plus and *CTC1* variants, one group found affected individuals had age-adjusted lymphocyte telomere length below the first percentile, as determined by flow FISH [18], whereas the other group found no difference in the relative leukocyte telomere length between affected and control individuals, as determined by qPCR [19]. Similarly, a report describing a patient with biallelic *CTC1* variants and classic DC with intracranial calcifications and non-specific vascular retinal changes, found very short lymphocyte telomere length by flow FISH [64]. In contrast, another report describing six individuals with DC or related bone marrow failure disorders and *CTC1* variants found no difference in relative telomere lengths between the affected individuals and controls. However,

these measurements were by qPCR [65]. Simultaneous measurements of telomere length using both methods in individual Coats plus and DC patient samples may ultimately resolve this question.

Distinguishing Revesz Syndrome from Coats Plus

As evident from the above descriptions, RS and Coats plus share several features: intrauterine growth restriction, bilateral exudative retinopathy, intracranial calcifications, sparse hair, nail dystrophy, and cutaneous changes. Recent evidence suggests that telangiectatic gastrointestinal bleeding, which was previously noted to be a feature of Coats plus, may be observed in some patients with RS [67]. However, they are distinct both clinically and genetically. Severe bone marrow failure is a dominant feature of RS, whereas this is not frequently described in patients with Coats plus. Patients with RS frequently have cerebellar hypoplasia, which is rare in Coats plus. Conversely, patients with Coats plus have a very distinctive pattern of intracranial calcification. Further, a skeletal phenotype of osteoporosis and easy fracture are common. Genetically, *TINF2* pathogenic variants are associated with RS [59, 60] whereas *CTC1* and *STN1* pathogenic variants are associated with Coats plus [18, 20]. Thus, the clinical features should lead to direct testing for variants in *TINF2* versus *CTC1* and *STN1*.

Isolated Aplastic Anemia, Myelodysplastic Syndrome, and Acute Leukemia

Aplastic anemia associated with very short lymphocyte telomere lengths or with a pathogenic variant in a telomere biology gene should raise suspicion for a TBD even in the absence of other features of DC. In young children, aplastic anemia may be the first manifestation of DC. As these children age, they may develop additional clinical features, including the mucocutaneous features characteristic of DC. In contrast, there are individuals who are well into adulthood when they develop aplastic anemia as the sole manifestation of a TBD. Notably, variants in *TERC* or *TERT*, and finding of short

telomeres have been reported in isolated adult cases of aplastic anemia, as well as in up to 5 to 10% of individuals in cohorts of seemingly acquired severe aplastic anemia [3, 68-70]. Although the reported individuals lacked physical features of DC, many had relatives who were also pathogenic variant carriers and had histories of macrocytosis, blood count abnormalities including aplastic anemia, myelodysplastic syndrome, or leukemia. Immunosuppressive therapy, which is typically effective in immune-aplastic anemia, was ineffective in these cases. Thus, a thorough family history and telomere length testing is recommended not only for children, but also adults with newly diagnosed aplastic anemia.

Similarly, rare germline variants in TBD genes, such as *TERT* and *TERC*, have been identified in sporadic adult cases of myelodysplastic syndrome and acute myeloid leukemia and in kinships predominantly manifesting these myeloid neoplasms in the absence of other features of DC [71-75]. Thus, as with patients with aplastic anemia, a thorough family history of patients with myeloid malignancy may reveal similarly afflicted relatives or those with varying degrees of bone marrow failure, which would prompt consideration of a familial telomerase or other telomere maintenance gene variant that presents predominantly as an isolated hematologic phenotype.

Telomere-Mediated Pulmonary Fibrosis

Pulmonary fibrosis (PF) is the most common manifestation of disease due to shortened telomeres [76], and up to 25% of familial cases and up to 10% of sporadic, idopathic cases are associated with a rare variant in a TBD gene, *TERT*, *TERC*, *DKC1*, *TINF2*, *RTEL1*, or *PARN* [24, 77-89]. In addition, variants in *NAF1* have been found in association with PF-emphysema [90]. The reader is referred to Chapter 14, Pulmonary Fibrosis, where PF and other TBD-associated lung disease are discussed in more detail. In brief, PF cases due to TBD gene pathogenic variants generally present in mid-adulthood. The majority are familial. The pedigrees of some familial cases are characterized by PF as the predominant phenotype [91], whereas other pedigrees evolve from a PF-predominant to bone marrow failure−predominant phenotype over successive generations [92]. The

presence of an underlying germline TBD gene pathogenic variant is highly suggested when PF is accompanied by cytopenias or other hematologic abnormalities such as macrocytosis or cryptogenic liver disease. Thus, thorough medical histories, examination of peripheral blood counts and liver function, and detailed family history are warranted with PF presentations.

Liver Disease-Predominant Phenotype

Similar to familial PF, pedigrees with a liver disease-predominant phenotype, as well as individuals with sporadic cryptogenic liver disease have been described with germline *TERT*, *TERC*, or *RTEL1* pathogenic variants [93-96]. The reader is referred to Chapter 18, Hepatic Complications, which describes in detail the hepatic manifestations associated with the TBDs. Here, we emphasize the importance of taking a thorough family history focused not only on familial liver disease, but also surveying for bone marrow and lung disease as steps in uncovering these cases [93].

Silent Carriers

Uncovering a pathogenic variant in an individual with clinical features of a TBD has the potential to lead to genetic testing and the discovery of additional family members who carry the variant but are asymptomatic, so-called silent carriers. The ability to anticipate the likelihood of developing disease or having offspring with disease may vary from relatively easy (as for a newborn male sibling with a pathogenic *DKC1* variant, who would be likely to develop disease) to more difficult (as for the highly unpredictable occurrence of myelodysplastic syndrome at 40 years of age in the offspring of a 60-year-old with a pathogenic *TERT* variant). Even more difficult are cases in which a familial variant is not identified, but testing revealed telomere lengths around the first percentile in asymptomatic relatives. As discussed in Chapter 5, Genetic Counseling, knowledge of silent carrier status may impact health-related behaviors (like avoidance of smoking or alcohol use), facilitate decisions on pre-implantation genetic counseling, and lead to disease surveillance (as with periodic CBCs or oral, head, and neck exams).

Lastly, silent carrier status would have significant implications with respect to related hematopoietic cell donation as such carriers would be unsuitable donors.

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Chapter 4

The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders

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Introduction

Pathogenic germline variants, also called mutations, in genes essential for telomere biology are the primary cause of dyskeratosis congenita (DC) and related Telomere Biology Disorders (TBDs) and can be inherited in X-linked recessive (XLR), autosomal dominant (AD), or autosomal recessive (AR) patterns (see Chapter 5, Genetic Counseling for Families). *De novo* germline mutations are also relatively frequent in DC/TBDs. To date, about 70-80% of patients with DC/TBDs have an identifiable pathogenic variant [1-3]. Currently, germline mutations in sixteen different telomere biology genes have been shown to cause DC/TBDs

(Figure 1) [1-4]. Seven of them are well-established disease genes (*DKC1*, *TERT*, *TERC*, *TINF2*, *CTC1*, *RTEL1* and *PARN*) while nine have so far been reported in only a small number of families (*NOP10*, *NHP2*, *WRAP53*, *ACD*, *POT1*, *STN1*, *NAF1*, *ZCCHC8* and *MDM4*). Genetic variants in *USB1* have been reported in patients with symptoms similar to those of DC/TBDs but with normal telomere lengths and more recently, variants in NPM1 that affect a modification of ribosomal RNA, have been reported in two DC/TBD patients. Most of the DC/TBD-associated pathogenic variants reported occur in a single patient and/or their family members (*i.e.*, a private mutation). However, a few do occur repetitively in multiple unrelated patients, most notably p.Ala353Val in *DKC1* (more than 40 families), p.Arg282His in *TINF2* (more than 30 families), and p.Arg1264His in *RTEL1* (a founder mutation in people of Ashkenazi Jewish ancestry) [5, 6]. As described elsewhere, there is a wide range of phenotypes associated with pathogenic variants in these genes (Table 1).

Figure 1: Schematic of the telomere and functions of the proteins affected in dyskeratosis congenita and the related telomere biology disorders. Yellow – autosomal dominant, blue – autosomal recessive, green – autosomal dominant or recessive, red – X-linked recessive, gray – not yet disease-associated. DKC1: dyskerin
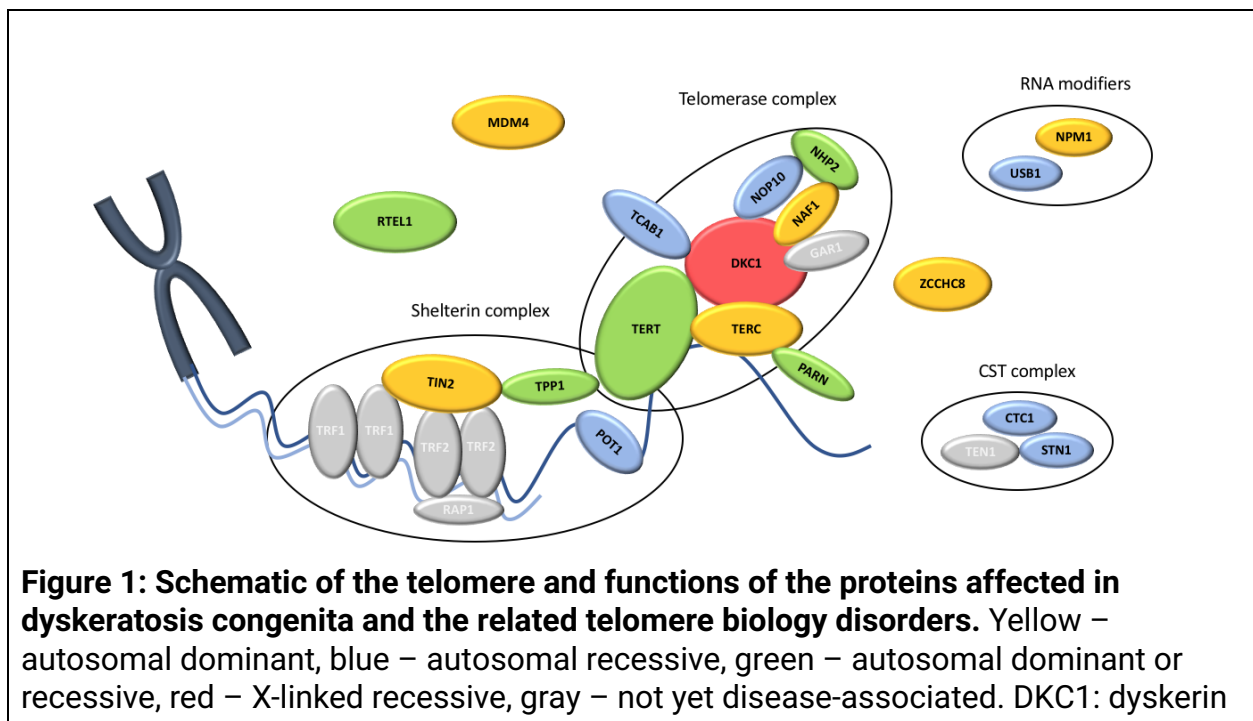
(encoding gene *DKC1*); TERC: hTR, human telomerase RNA component (*TERC*); TERT: human telomerase reverse transcriptase (*TERT*); NOP10: nuclear protein family A, member 3 (*NOP10*); NHP2: NOLA2 nucleolar protein family A, member 2 (NHP2); NAF1: nuclear assembly factor 1 ribonucleoprotein (*NAF1*); GAR1: nucleolar protein family A, member 1 (*GAR1*); PARN: poly (A)-specific ribonuclease (*PARN*); TCAB1: telomere Cajal body associated protein 1 (*WRAP53*); TPP1: telomere protection protein 1 (*ACD*); STN1: CST complex subunit (*STN1*); CTC1: conserved telomere maintenance component 1 (*CTC1*); RTEL1: Regulator of telomere elongation helicase 1 (*RTEL1*); TIN2: TERF1 interacting nuclear factor 2 (*TINF2*); TRF1: telomeric repeat binding factor 1 (*TERF1*); TRF2: telomeric repeat binding factor 2 (*TERF2*); RAP1: TERF2 interacting protein (*RAP1*).
Components are grouped based on their function.

Telomerase-Associated Genes

The first DC-associated gene, XLR *DKC1*, was discovered by linkage analysis in 1998 [7]. The protein encoded by this gene, called dyskerin, was known by homology to be involved in the maturation of ribosomal RNA. The connection between DC/TBDs and telomere length was made when dyskerin was shown to affect telomerase RNA. Primary fibroblasts (skin cells) and lymphoblasts (made from lymphocytes, a type of white blood cell) from patients with DC bearing *DKC1* mutations exhibited low levels of telomerase RNA, reduced telomerase activity, and short telomeres compared to normal controls [8].

The link between DC/TBDs and telomere biology was supported by the subsequent discovery of pathogenic variants in hTERT or hTR (encoded by *TERT* and *TERC*, respectively) in patients with AD forms of DC/TBDs [9, 10]. The *TERT* variants found in these patients are generally nonsynonymous coding mutations that lead to reduction of telomerase activity due to changes in key amino acids. *TERC* encodes the RNA template required for the addition of the (TTTAGG)n telomeric DNA nucleotide repeats by telomerase. In addition to mutations affecting the template region of *TERC*, mutations in other domains as well as the promoter region of *TERC* have been described [11]. Rarely, *TERT* can be a cause of AR forms of DC/TBD; biallelic pathogenic variants are

associated with more severe disease and patients have dramatically reduced levels of telomerase. AR DC/TBD can also be the result of biallelic mutations in *NOP10* or *NHP2*, (encoded by genes of the same names), both of which affect telomerase biogenesis, while heterozygous mutations in *NHP2* have been reported in families with AD pulmonary fibrosis (PF) [12-14]. Loss of function variants in *NAF1*, another factor involved in telomerase biogenesis, have been found in two families presenting with AD inheritance of DC/TBD and affected individuals diagnosed with PF, myelodysplastic syndrome (MDS), and/or liver disease [15].

Disruption of telomerase trafficking in the nucleus can result from germline mutations in TCAB1 (encoded by *WRAP53*) [10]. Patients with compound heterozygous mutations in TCAB1 were reported to have features of classic DC. Their relatives who had one mutant allele had normal telomere lengths, suggesting that biallelic mutations are required for clinical manifestations. Compound heterozygous mutations in patient cells prevented telomerase from localizing to Cajal bodies for assembly. This results in misdirection of telomerase RNA to the nucleoli and precludes telomerase from elongating telomeres.

Defects in the maturation of hTR can also cause DC/TBDs. Bialleic mutations in PARN, a deadenylase which mediates 3'-end processing of hTR, were shown to cause severe early onset DC resembling the Hoyeraal Hreidarsson syndrome (HH) [16-19]. Heterozygous mutations in PARN, on the other hand, are associated with AD inheritance of PF [20]. More recently, a mutation ZCCHC8, which is also required for hTR 3'-end maturation, has been found in a family with AD PF [21].

The Shelterin Telomere Protection Complex

Germline pathogenic variants in TIN2 (encoded by *TINF2*) are also responsible for AD DC, mostly occurring *de novo* [22, 23]. TIN2 is not directly involved in telomerase function; rather, it is part of the shelterin complex, a six-protein telomere-specific complex that protects telomeres and participates in length regulation. Causative *TINF2*

mutations cluster at the consensus site for heterochromatin protein 1-gamma (HP1ɣ). This association between *TINF2* and HP1ɣ is required for sister telomere cohesion, thereby preventing sister telomere loss [24].

Pathogenic variants in two other shelterin components, *ACD* (encoding TPP1), and *POT1* (encodes POT1), have been described in rare patients with TBDs. Patients with *ACD* variants presented with variable phenotypes, including HH with AR inheritance and aplastic anemia/myelodysplasia with AD inheritance [25, 26]. *POT1* biallelic variants were identified in a patient with Coats plus [27].

Telomere Capping Proteins

Compound heterozygous pathogenic variants in CTC1 were first reported as a cause of Coats plus and in a clinically similar disorder termed cranioretinal microangiopathy with calcifications and cysts [28, 29]. Patients with those mutations had short telomeres and features that phenotypically overlapped with DC/TBDs [28-30]. Mutations in *CTC1* were subsequently demonstrated to cause AR DC/TBDs [31, 32]. Patient telomere length in *CTC1*-associated DC/TBD was not as short as in DC/TBD due to other causes, but still shorter than unaffected individuals. *CTC1* encodes part of the CST complex along with *STN1* and *TEN1*. Two individuals with Coats plus due to pathogenic germline variants in *STN1* have also been described [33]. The CST complex has both extra-telomeric and telomeric roles; at the telomere, it cooperates with the shelterin complex to protect telomeres from degradation and aberrant recognition by DNA repair machinery [34].

Regulator of Telomere Elongation Helicase 1 (*RTEL1*)

Several groups independently identified *RTEL1* mutations using whole exome sequencing in families with DC and HH [35-37]. The RTEL1 protein regulates telomere length, may interact with PCNA (proliferating cell nuclear antigen), and also plays a role in DNA repair [4, 38]. Most of the *RTEL1* mutations appear to be AR, but AD mutations

have also been reported, particularly in patients presenting with late onset pulmonary disease [14, 39]. As many as 1 in 100 to 1 in 200 individuals of Ashkenazi Jewish ancestry may carry the RTEL1 p.Arg1264His founder mutation, which has led to the inclusion of *RTEL1* in prenatal genetic testing panels for this population [6, 40, 41].

U6 Small Nuclear RNA Biogenesis 1 (*USB1*)

Linkage analysis led to the identification of mutations in *C16orf57*, which at the time was of unknown function [27]. It is now called *USB1* and known to be involved in the maturation of a small nuclear RNA (U6), which plays a crucial role in RNA splicing. *USB1* mutations were first reported in individuals with Rothmund Thomson syndrome and poikiloderma with neutropenia, suggesting an overlapping clinical spectrum [42]. These patients, including those with a DC/TBD phenotype, tend to have normal telomere lengths. However, it is interesting to note that yeast cells which lack the orthologue of this protein (*Δmpn1*) display increased levels of telomeric repeat-containing RNA and short telomeres [29].

MDM4 Regulator of P53

MDM4 is a key regulator of the tumor suppressor protein p53 (encoded by *TP53*). AD inheritance of a pathogenic variant in *MDM4* was discovered in a family with a history of bone marrow failure, early-onset head and neck squamous cell carcinoma, and short telomeres. Laboratory and animal model studies showed that the *MDM4* variant activated the p53 pathway which, in turn, caused telomere dysfunction [43].

Table 1. Genes associated with DC/TBDs.

Gene	Gene Product	Patterns of Inheritance	Role	Predominant Phenotypes
ACD	TPP1	AD, AR	Shelterin component	DC-like
CTC1	CTC1	AR	Telomere extension	DC, CP, CM
DKC1	Dyskerin	XLR	Telomerase component	DC, HHS
MDM4	MDM4	AD	Telomere associated regulation of p53	DC-like
NAF1	NAF1	AD	Telomerase component	PF
NHP2	NHP2	AR, AD	Telomerase component	DC, PF
NOP10	NOP10	AR	Telomerase component	DC
NPM1	NPM1	AD	No telomere association (rRNA modification)	DC
PARN	PARN	AR, AD	hTR maturation	DC, HHS, PF
POT1	POT1	AR	Shelterin component	CP
RTEL1	RTEL1	AR, AD	Telomere replication	DC, HHS, PF, MDS, LC
STN1	STN1	AR	Telomere extension	CP
TERC	hTR	AD	Telomerase component	DC, AA, MDS, PF
TERT	hTERT	AD, AR	Telomerase component	DC, AA, MDS, PF, LC

TINF2	TIN2	AD	Shelterin component	DC
USB1	USB1	AR	No telomere association (snRNA maturation)	DC-like, PN, RTS
WRAP53	TCAB1	AR	Telomerase component	DC
ZCCHC8	ZCCHC8	AD	hTR maturation	PF

Abbreviations: DC, dyskeratosis congenita; HHS, Hoyeraal Hreidarsson syndrome; AA, aplastic anaemia; MDS, myelodysplastic syndrome;
AML, acute myeloid leukaemia; PF, pulmonary fibrosis; LC, liver cirrhosis; RS, Revesz syndrome; CR, Coat's retinopathy/plus; CM, cerebroretinal microangiopathy with calcification and cysts; PN, poikiloderma with neutropenia; RTS, Rothmund-Thomson syndrome.

Genetic Heterogeneity

Our understanding of the genetic causes of DC/TBDs is complicated by the presence of silent carriers and variable clinical manifestations that progress with time. This variability results in incomplete clinical penetrance of disease-associated pathogenic variants. Incomplete penetrance occurs in genetic disorders when a person with a disease-associated mutation does not develop the expected clinical features. This is possibly due to a combination of genetic, environmental, and lifestyle factors. As more family members are tested for TBD-associated mutations, more silent carriers are being recognized. Specifically, carriers of germline mutations in *TERT*, *TERC*, *RTEL1*, and *PARN* with few symptoms consistent with DC/TBDs have been identified because of the increased scrutiny brought about by the diagnosis of a family member. This occurs at least in part because the clinical signs and symptoms of DC/TBDs can develop at different rates in different individuals, even within the same family. For example, the phenotype of very short telomeres (less than the first percentile for age – 99 of 100 people of the same age have longer telomeres) in individuals from a family with variable

clinical penetrance was used in the linkage scan that discovered mutations in *TINF2* as a cause of DC [30]. Silent carriers of DC/TBD-associated mutations should be counseled regarding their potential risk of disease.

As next generation sequencing technologies become widely available to aid in the molecular diagnosis of DC/TBDs, the number of genetic variants of unknown significance identified increases significantly. The status of these variants remains uncertain until further laboratory, population, and/or family studies are conducted to determine whether they are clinically significant or benign. This is true for all rare diseases and as a result, more stringent criteria have been introduced in order to assign pathogenic status to novel variants. In genes such as *TERT* where private (occurring in one family only) missense (amino acid changing) mutations with variable penetrance are the most common cause of disease, new sporadic variants will often not meet sufficient criteria to be classified as pathogenic.

Genetic anticipation refers to a younger age of onset and increased severity of the symptoms of a disease over successive generations within a family. This has been reported in cases of telomerase haploinsufficiency: older generations are often asymptomatic or may have adult-onset pulmonary fibrosis and/or liver disease, but later generations with the same mutation can exhibit classic symptoms of DC or present with aplastic anemia in childhood [9, 44, 45]. A similar finding has been noted in a family with a *TINF2* mutation [46]. It is also notable that in all of these reports the offspring have shorter telomeres than the parents.

Genetic analysis of DC/TBDs is made more complex by the recent identification of somatic mosaic reversion. This phenomenon has been reported in DC families where a germline *TERC* mutation identified in skin fibroblasts was spontaneously corrected by mitotic recombination in blood cells [33].

Summary

Causative germline pathogenic variants (*i.e.*, mutations) have been identified in about 70-80% of patients with DC/TBDs. Scientists are using next-generation sequencing technologies to discover the genetic cause of DC/TBDs in mutation-negative families. Genetic counseling for the patient and their family members is an integral component of DC/TBD clinical management (see Chapter 5, Genetic Counseling for Families). This can be particularly challenging in the context of the variable penetrance and variants of unknown significance, discussed above.

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Chapter 5

Genetic Counseling for Families

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Introduction

The National Society of Genetic Counselors defines genetic counseling as the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease (*National Society of Genetic Counselors*, http://www.nsgc.org; *About Genetic Counselors*, https://www.aboutgeneticcounselors.org/).

The process of genetic counseling integrates the interpretation of family and personal medical histories to assess the chance of disease occurrence or recurrence. Genetic counseling may include a discussion of the diagnosis of and/or screening for inherited disease(s), types of inheritance, available genetic testing options, genetic test results, disease management, referral to genetic disease support groups and/or other resources, as well as potential research

opportunities.

Genetic counselors are generally master's degree level healthcare professionals who typically work as part of a medical care team. An appointment with a genetic counselor may provide individuals and families with a better understanding of a telomere biology disease (TBD) diagnosis through discussions including family history, clinical findings, general and TBD-specific genetic information, genetic testing options and results, psychological and social issues, risk assessment for other family members, family planning, and identification of support options for families with a TBD. A local genetic counselor can be found through the findageneticcounselor.nsgc.org website.

Overview of the Genetics of Telomere Biology

Our cells contain all of our genetic material, called DNA, in 23 pairs of chromosomes. Chromosome pairs 1-22 are autosomal meaning they are the same between males and females. The last pair of chromosomes are called the sex chromosomes. Males have an X and a Y sex chromosome while females have two X chromosomes. Individual genetic instructions in our DNA are called genes. Genes encode all the instructions needed for our bodies to function properly. The genes in our DNA are made up of four bases called adenine (A), guanine (G), thymine (T), and cytosine (C). Combinations of these bases in groupings of three encode twenty amino acids. Amino acids are assembled together, like beads on a string, to make proteins. Proteins contain the information necessary for cells to perform their specific functions.

If the A, T, G, and C bases of the amino acid code are changed, the protein will not be assembled or function properly. Changes in the gene are called variants (historically called mutations). In the case of genes associated with TBD, the variants affect proteins

important in maintaining the ends of our chromosomes called telomeres. Telomeres are essential for the stability of our chromosomes, thus individuals with TBD-associated genetic variant(s) will most often have shorter than normal telomeres for an individual of their age (see also Chapter 2, Why Telomeres Matter).

The Genetics & Inheritance of TBD

As of March 2021, there are 15 established genes known to be associated with TBDs: *ACD*, *CTC1*, *DKC1*, *NAF1*, *NHP2*, *NOP10*, *PARN*, *POT1*, *RTEL1*, *STN1*, *TERC*, *TERT*, *TINF2*, *WRAP53*, and *ZCCHC8*. There are other genes for which there is limited evidence for an association with TBDs: *DCLRE1B/SMN1B/Apollo*, *GRHL2*, *LIG4*, *MDM4*, *NPM1*, *RPA1*, and *USB1*. At least 70% of individuals who meet clinical diagnostic criteria for DC have a disease-causing variant in one of the established genes [1].

TBDs can be inherited in an autosomal or X-linked pattern. Both males and females have two copies of autosomal genes. Autosomal dominant (AD) inheritance means that an individual only needs a disease-causing variant on one copy of an autosomal gene to have a TBD. Each child of an individual with an AD variant has a 50% chance of inheriting the variant from that parent (Table 1).

Autosomal recessive (AR) inheritance means that an individual has a TBD from having a disease-causing variant on both copies of the same autosomal gene associated with TBD. When a child has an AR form of TBD, each of their parents is typically a "carrier" of one of the disease-causing variants identified in the child. In each pregnancy for two carriers together, there is a 25% chance to have a child inherit both variants and have a TBD, a 50% chance to have a child who inherits one variant and is a carrier of TBD, and a 25% chance of having a child who does not inherit either variant. For two individuals to have a child with AR TBD, they must both carry a variant in the same TBD-associated gene (Table 1).

Some of the TBD autosomal genes can be associated with both autosomal dominant

and autosomal recessive disease (Table 1). Typically, individuals with a disease-causing variant in one copy of the TBD-associated gene (AD) have milder disease than individuals with a disease-causing variant on both copies of the same gene (AR).

While the majority of the genes associated with TBDs are autosomal, one TBD-associated gene called *DKC1* is located on the X chromosome and is inherited in an X-linked (XL) pattern (see Table 1). Since female sex chromosomes are XX, they have two X chromosomes and two copies of the *DKC1* gene. Since male sex chromosomes are XY, they only have one X chromosome and only one copy of the *DKC1* gene. Males who have a disease-causing variant on their only copy of the *DKC1* gene are expected to have symptoms since they no longer have any functioning *DKC1*. Females with a disease-causing variant on one of their two copies of *DKC1* gene tend to be either unaffected or have more mild symptoms because they have a second, working copy of *DKC1*. Historically, females with a *DKC1* gene variant were referred to as "carriers" as they have an increased chance of having a child with a TBD. This term has faced some opposition, as it does not account for the symptoms that some women with this form of TBD may experience.

Any child of a female with an XL variant will have a 50% chance of inheriting the X chromosome with the variant. Males who inherit the variant are expected to have disease symptoms while females who inherit the variant could have an increased chance of developing some symptoms in their lifetime. Each daughter has a 50% chance of inheriting the X chromosome with the variant. Males with XL TBD will pass the variant to all of their daughters (who inherit the X chromosome with the *DKC1* gene variant) and none of their sons (who inherit the Y chromosome).

In the majority of cases, the variant(s) that is causing the TBD symptoms in an individual was inherited from one or both parents. However, in some cases the disease-causing variant was new in the individual with a TBD. This is called a *de novo* variant. If a TBD-associated variant is found in a proband but not found in a parent, it is considered likely *de novo*. Due to the small chance of gonadal mosaicism in a parent

with negative testing, there remains a small residual chance of disease recurrence in a future pregnancy.

Table 1: Mode of Inheritance of TBD and Possible TBD Associated Genes

Mode of Inheritance	TBD Gene(s)
X-linked (XL)	*DKC1*
Autosomal Dominant (AD)	*NAF1, TERC, TINF2, ZCCHC8*
Autosomal Dominant (AD) or Autosomal Recessive (AR)	*ACD, NHP2, PARN, RTEL1, TERT*
Autosomal Recessive (AR)	*CTC1, NOP10, POT1, STN1* (*OBFC1*), *WRAP53*

Genotype/Phenotype Correlations and Symptom Variability

The variant(s) and the TBD-associated gene that is impacted is called the genotype. The phenotype refers to the clinical symptoms identified in an individual. Genotype/phenotype correlations refers to the association between the possible or likely clinical symptoms based on the identified genotype. This section highlights some of the genotype/phenotype findings for various TBD-associated genes. It is important to know that even within individuals in the same family with the same genetic cause of TBD, the symptoms that each individual experiences and the age of symptom onset can vary. This is called variable expressivity. Further, not everyone with the gene variant associated with TBD may show symptoms of the disease. This is called reduced penetrance. Finally, in some families with TBD, subsequent generations may be more severely affected or have an earlier onset of disease than in previous generations. This is called anticipation.

These concepts help explain why the term telomere biology disorders, or TBD, is used in this second edition of the Clinical Guidelines instead of dyskeratosis congenita. While historically dyskeratosis congenita was defined by the classic clinical triad (see Chapters 3, Diagnosing TBDs & Subtypes of TBDsand 6, Dermatologic Manifestations), it is now known that the spectrum of disease extends from pediatric patients with classic presentations to individuals presenting with a single symptom, such as pulmonary fibrosis, in adulthood. Based on this understanding, the term TBD is used to describe families with telomere disease, as it encompasses the full spectrum of symptoms in disorders of telomere maintenance.

ACD: Heterozygous (AD) and compound heterozygous (AR) variants in *ACD* have been reported. A three generation family with a heterozygous *ACD* variant presented with short telomeres, bone marrow failure, and oral cancer without dysmorphism, mucocutaneous manifestations, or anomalies of the skeletal, cardiac or urogenital system [2]. A compound heterozygous case was reported with clinical features consistent with Hoyeraal Hreidarsson syndrome including microcephaly, cerebellar hypoplasia, developmental delay, oral leukoplakia, nail dystrophy, and esophageal stenosis [3].

CTC1: Homozygous and compound heterozygous variants (both AR) in *CTC1* have been associated with Coats Plus and less commonly with classic dyskeratosis congenita [4-7]. Limited information is available on possible disease symptoms in carriers [8]. Some reports have demonstrated telomere lengths at or less than the first percentile for age in homozygous/compound heterozygous cases while heterozygous relatives had telomere lengths on the lower end of normal [7, 9]. Others have reported normal telomere length studies in cases, carriers and controls [5, 6].

DKC1: Males with a hemizygous (XL) variant in the *DKC1* gene can present with a wide range of clinical phenotypes. The classic triad is commonly seen in males with *DKC1* variants. The classic dyskeratosis congenita and Hoyeraal Hreidarsson syndrome phenotypes predominate; however, more isolated disease symptoms or later onset

disease severity has been reported [10-13]. Females with *DKC1* (previously called carriers) can be asymptomatic or show more mild disease symptoms such as features of the classic triad, dental anomalies, hair abnormalities although some isolated cases of women with more pronounced disease symptoms have been reported [14-17].

NAF1: Heterozygous (AD) frameshift variants have been identified in individuals with short telomere length, pulmonary fibrosis, and bone marrow failure [18].

NHP2: Homozygous and compound heterozygous variants (both AR) in *NHP2* were originally reported in cases with classic dyskeratosis congenita findings [19, 20]. A subsequent publication demonstrated a compound heterozygous case with the Hoyeraal Hreidarsson syndrome phenotype including intrauterine growth retardation, microcephaly, cerebellar hypoplasia, developmental disability, lymphopenia, and the classic triad. In addition, multiple heterozygous (AD) cases were reported with aplastic anemia, interstitial lung disease, and pulmonary fibrosis. While both the individuals with apparent autosomal recessive and autosomal dominant disease had telomere lengths at or less than the 1st percentile for age, the parents of the child with HH who are heterozygotes had normal telomeres and were reportedly asymptomatic in young adulthood [21].

NOP10: A homozygous (AR) variant in *NOP10* was reported in three siblings with reticular skin pigmentation and nail dystrophy without leukoplakia. Other symptoms included abnormal dentition, thickening of the skin on the palms and soles, and short telomeres. One individual had hypocellular bone marrow and pancytopenia. Heterozygous relatives had telomeres that were significantly shorter than controls but not as short as the homozygous cases [22]. A more recent report identified two second cousins once-removed with the same homozygous variant in the *NOP10* gene presenting in infancy with cataracts, hearing impairment, nephrotic syndrome, enterocolitis and short telomeres. One child was also found to have cerebellar hypoplasia and hypomyelination. One relative with a heterozygous variant had hearing

impairment [23].

PARN: Homozygous/compound heterozygous (both AR) and heterozygous (AD) variants in *PARN* present with a severe phenotype in some individuals, while others have pulmonary fibrosis. Clinical findings may include short telomeres [24] and immunologic abnormalities [25], as well as intrauterine growth retardation, microcephaly, central nervous system calcifications, severe developmental delay, cerebellar hypoplasia, esophageal and urethral stenosis, hip avascular necrosis, bone marrow failure abnormal skin pigmentation, oral leukoplakia, nail dysplasia, hyperkeratosis of palms and soles, and multiple fractures [26, 27].

POT1: Homozygous (AR) variants in the *POT1* gene have been associated with a very early onset and rapidly progressing form of Coats Plus (CP). The reported symptoms included intrauterine growth retardation, leukoencephalopathy, gastrointestinal ectasia, bone fracture, developmental disabilities, and sparse hair in addition to the intracranial calcifications and retinal exudates that are typical of CP [28]. Heterozygous (AD) variants in *POT1* have also been associated with an increased risk of developing certain malignancies [29].

RTEL1: Heterozygous (AD) and homozygous/compound heterozygous (both AR) variants have been reported in *RTEL1* in association with short telomeres and disease. A founder *RTEL1* variant, R1264H, has been identified in the Ashkenazi Jewish population [30, 31]. Clinical findings for individuals with homozygous or compound heterozygous variants may be suggestive of DC or Hoyeraal Hreidarsson syndrome and may be more severe than those with heterozygous variants [31-33]. Multiple families with heterozygous variants in *RTEL1* have presented with adult-onset disease, often with an isolated feature such as pulmonary fibrosis, liver disease, or bone marrow failure [32-35]. Importantly, these phenotypes are not necessarily uniform in families, and other disease features may be seen, including the dyskeratosis congenita phenotype [36-37].

STN1: Two patients with homozygous (AR) variants in the *STN1* gene have been

reported with a Coats Plus phenotype. Symptoms included intrauterine growth retardation, premature greying, poor growth, liver fibrosis, portal hypertension, esophageal varices, brain calcifications with white matter changes, osteopenia, gastrointestinal hemorrhage, telangiectasia, and pancytopenia with hypocellular bone marrow. One patient developed significant neurologic disease including spasticity, dystonia, and ataxia. One of the reported cases had normal telomere lengths while the other had short telomeres [38].

TERC: Variants in the *TERC* gene have been associated with a wide phenotypic spectrum from unaffected adults to pediatric onset disease. Some individuals present with isolated features of telomere disease such as lung, liver or hematologic abnormalities in later adulthood while others present with more classic dyskeratosis congenita symptoms with some or all of the classic triad including a possible case with the Hoyeraal Hreidarsson phenotype [39-43]. While telomere lengths are often less than the first percentile for age, some affected individuals have telomere lengths that are more moderately short [44-46]. While TERC is associated with an autosomal dominant form of TBD, a case of more pronounced disease in an individual with two *TERC* gene variants has been reported [47].

TERT: Heterozygous (AD) and homozygous/compound heterozygous (both AR) variants in the *TERT* gene have been associated with disease symptoms. Some individuals with one variant in *TERT* may have classical DC findings such as the clinical triad, bone marrow failure, cancer, and liver cirrhosis [46, 48, 49]. However, others may only have an isolated disease symptom such as pulmonary fibrosis [50] or be asymptomatic. Additionally, those with homozygous/compound heterozygous *TERT* variants may have a severe Hoyeraal-Hreidarsson phenotype [51]. As with the *TERC* gene, telomere lengths are often less than the first percentile for age, some affected individuals have telomere lengths that are more moderately short [44-46].

TINF2: Variants in the *TINF2* gene are heterozygous (AD), often identified in exon 6 of the gene, and commonly *de novo*. Patients with *TINF2* variants usually have very short

telomeres. Clinical findings may be severe (some *TINF2* variants have been identified in those with Hoyeraal Hreidarsson or Revesz syndromes) and include aplastic anemia, bone marrow failure, dystrophic nails, skin abnormalities, leukoplakia, oral cancer, osteoporosis, avascular necrosis of the hip, epiphora, pulmonary fibrosis, gastrointestinal hemorrhage, bilateral exudative retinopathy, intracranial calcification, microcephaly, developmental delay, and immune deficiency [49, 52, 53].

WRAP53: Patients with compound heterozygous (AR) variants in the *WRAP53* gene have been reported with classic dyskeratosis congenita symptoms including the classic triad, bone marrow failure, and telomere lengths less than the 1st percentile for age [54, 55]. In addition, *WRAP53* variants have been reported in association with the Hoyeraal-Hreidarsson variant of telomere disease including cerebellar hypoplasia, developmental disabilities, microcephaly, intrauterine growth retardation, hypotonia, gastrointestinal abnormalities and progressive bone marrow failure among other findings [55]. Heterozygotes were reported as healthy with normal telomere lengths.

ZCCHC8: Heterozygous (AD) variants in the *ZCCHC8* gene have been associated with pulmonary fibrosis, bone marrow failure and short telomeres [56].

Genotype/Phenotype for Possible TBD-Associated Genes

DCLRE1B/SMN1B/Apollo: A patient with clinical features of Hoyeraal-Hreidarsson syndrome (severe intrauterine growth retardation, microcephaly, cerebellar hypoplasia, lack of B lymphocytes, progressive aplastic anemia, severe enteropathy, severe bone marrow failure) was identified as having a heterozygous (AD) variant in *Apollo* [57].

GRHL2: Two consanguineous families with an original diagnosis of dyskeratosis congenita were found to have different homozygous (AR) variants in the *GRHL2* gene. Variants in *GRHL2* have been associated with ectodermal dysplasia/short stature syndrome with clinical features that overlap with TBD including nail dystrophy, abnormal oral pigmentation, and keratoderma and hyperkeratosis of the hands and feet. Telomere

lengths were not reported to be short [58].

LIG4: Two families with an original diagnosis of dyskeratosis congenita were found to have compound heterozygous (AR) variants in the *LIG4* gene. Variants in *LIG4* have been associated with *LIG4*/Dubowitz syndrome. Some overlapping features of TBD and *LIG4/*Dubowitz include bone marrow failure, various skin abnormalities, immune deficiency, microcephaly, and developmental delay. Telomere lengths were not reported to be short [58].

MDM4: One report identified four heterozygous (AD) *MDM4* gene cases and one obligate carrier in one family. Phenotypes included neutropenia, bone marrow hypocellularity, AML, HNSCC, B cell deficiency, vague gastrointestinal symptoms, and chronic pain [59].

NPM1: Two patients have been reported with heterozygous (AD) *NPM1* variants located in the same region of the gene. One patient had severe growth defects at birth, thumb abnormalities and thrombocytopenia, and the other had skin pigmentation abnormalities, nail dystrophy, microcephaly, developmental delay, short stature, skeletal abnormalities in the radius, and bone marrow failure [60].

RPA1: A child presenting with pancytopenia, hypocellular bone marrow, the mucocutaneous triad, a congenital renal anomaly, and short telomeres had a *de novo* heterozygous (AD) variant in *RPA1*. The allelic frequency of the variant was higher in fibroblasts than bone marrow, and two somatic compensatory events were identified, likely consistent with a gain-of-function effect [61].

USB1: Eight families with an original diagnosis of dyskeratosis congenita were found to have homozygous (AR) variants in the *USB1* gene. Variants in *USB1* have been associated with poikiloderma with neutropenia. Overlapping findings between TBD and poikiloderma with neutropenia include bone marrow failure, abnormal skin pigmentation and nail abnormalities. Telomere lengths were not reported to be short [58].

Evaluation and Testing

Family and Medical History

A family, individual medical, and cancer history is obtained to help determine whether only the individual being evaluated may have a TBD or whether other family members may also be at risk of having the disorder. In preparation for a genetics evaluation, a family should spend time thinking about relatives on both sides of the family: do any individuals in the family have any findings related to TBD or cancers? This may require speaking with other family members, since some of the features of a TBD are subtle and some individuals may be more private with their health information.

It is important to remember that not every individual with a TBD will have disease features. The symptoms in the family and the age that disease symptoms present may vary. The family history may help the healthcare team in deciding which family member(s) would be the most helpful to evaluate and/or offer testing. Testing the relatives of an individual known to have a TBD may identify other unsuspected family members who have the TBD-associated variant for whom genetic counseling would be beneficial.

Testing for TBD

Once the healthcare provider suspects the diagnosis of a TBD, a genetic counseling consultation with the individual and/or family can be helpful in explaining more about TBD, possible inheritance patterns, telomere and molecular genetic testing options, the testing process, and insurance concerns relating to genetic testing. Other concerns may be addressed more precisely once the variant for a specific gene is identified (see "Positive Results" below). Testing may be helpful for other family members to identify their chance of having a TBD or being a carrier of TBD based on the inheritance pattern of the TBD in the family. Individuals with the variant(s) identified in the family should be offered clinical consultation with a qualified medical provider to discuss the screening

recommendations for early detection and appropriate clinical care. (see Chapter 3, Diagnosing TBDs & Subtypes of TBDs.)

Telomere Length Measurements

The first step in testing for a suspected TBD is to assess the telomere length in specific subtypes of white blood cells. This test is very sensitive in screening for a TBD known to be associated with short telomeres (see Chapter 3, Diagnosing TBDs & Subtypes of TBDs). If all or nearly all of the white blood cells' telomere lengths (tests commonly evaluate either 2 or 6 subtypes of white blood cells) are determined to be very short (less than 1% length for their age), the test result is consistent with the diagnosis of a TBD. However, it is possible that not all individuals with a TBD will have all very short telomeres.

Genetic Testing Methodologies

Once an individual has been identified to have clinical features and/or telomere lengths that are consistent with or suggestive of a TBD, genetic testing is recommended for TBD-associated genes to try to identify a causative gene variant. Historically the term "mutation" was used for many years to refer to a change in the DNA that was thought to be responsible for causing genetic disease. All changes in the DNA are now referred to as "variants" and further described as one of five types of variants: pathogenic (disease causing), likely pathogenic, variant of uncertain significance, likely benign, or benign (does not cause disease). The interpretation of a variant may change over time as more information is known about a specific gene or variant or if additional information becomes available from familial testing. It is important to check in with the testing laboratory periodically as additional information on the familial variant may become available over time that impact the classification.

A genetic counseling session prior to testing for TBD provides individuals with an understanding of general and TBD-specific genetics concepts, as well as the process by which genetic testing occurs and results that may be identified. It is also an opportunity

to review the testing consent form, and discuss the risks, benefits and limitations of testing.

The decision regarding which TBD genetic test to offer is based on many factors such as whether or not a variant has been previously identified in a family member or whether there are other clinical findings that may suggest a larger set of genes should be evaluated (whole exome or whole genome sequencing).

Targeted testing is appropriate if a variant in a TBD gene has already been identified in a family member. Other family members can then be tested for the specific variant in the TBD gene previously found in the family. However, additional family and medical history should be evaluated prior to testing any individual to determine if there are other findings in them or their family members who are not related to the side of the family with the identified TBD variant that suggest that testing with a larger gene panel would be more appropriate.

The first individual in a family to be tested for TBD genes will most often have Next Generation Sequencing (NGS) panel testing where multiple TBD genes are tested at the same time. Many NGS panels do not test for all known TBD genes due to the constantly expanding number of TBD-associated genes. NGS panels should include sequencing and copy number variant (CNV) analysis of the TBD-associated genes. Analysis of genes by sequencing and CNV testing involve different technologies and look for different types of variants in genes.

Whole exome sequencing (WES) and/or whole genome sequencing (WGS) may be beneficial if an individual has a complex clinical phenotype with atypical findings or if the NGS TBD panel testing was negative. WES will sequence our exons and splice sites, which account for about 2% of our genome. WGS analyzes the whole genome, but currently the interpretation of variants identified in areas outside of the exons and splice sites is often limited. WES/WGS testing increases the complexity of testing, often requiring specimens from family members and creating a greater potential to uncover incidental or secondary findings. As with all genetic testing, comprehensive, informed

consent is needed prior to initiating testing.

Interpretation of Test Results

Positive Results

If a pathogenic or likely pathogenic variant(s) is identified, the results are considered positive or diagnostic for a TBD. The positive results are provided to the patient. This discussion typically includes an explanation of the specific gene variant and the associated gene, a review of the inheritance of the specific gene in their family, the reproductive implications of the finding, medical screening and management recommendations, and consideration for testing of other family members. The psychosocial implications of a TBD diagnosis are considered and additional resources for support are discussed.

Negative Results

When the clinical presentation and telomere length analysis in the individual is consistent with a TBD but no variant is found in a known TBD gene, an as yet unidentified TBD gene may be responsible. Once testing of the clinically available TBD genes has been completed, testing can be put on hold until additional TBD genes have been identified. Alternatively, WES/WGS or research study enrollment may help identify a novel or previously undetectable genetic cause of disease. Individuals with disease can continue to be managed based on their clinical diagnosis at the guidance of their medical team.

Variants of Uncertain Significance

A variant of uncertain significance (VUS) is a change in the DNA for which there is not enough current information to determine whether the variant alters how the gene works in the body and is related to disease. It may be that the variant is rare, or has never previously been identified. An assumption that it causes disease cannot be made from

the available evidence. If an individual has a VUS, their clinical care and management should be based on their personal and/or family history and not the VUS. Genetic testing technology is rapidly evolving and, in time, may lead to a more certain interpretation of variants currently identified as a VUS.

Familial Implications of Genetic Findings

It is important to realize that the results of genetic testing for an individual have health and reproductive implications for family members as well. Further, if the individual with a TBD is being considered for a bone marrow or organ transplant from a relative, testing to assure that the donor relative does not also have a TBD is critical.

After a positive test result, other family members can pursue testing targeting the familial variant, unless there are other indications to consider additional testing for that individual. Information on the specific gene and variant identified will determine which relatives could benefit from testing.

Testing can determine whether one or both parents of the individual have the variant(s), or if the variant is likely a new (*de novo)* variant in their child with a TBD. In rare cases, individuals with a child with a likely *de novo* variant will have another child with a TBD. This is due to germline mosaicism where a parent has a TBD-causing variant in their egg or sperm cells but not in the tissue used in their genetic studies for the TBD. Based on this information, negative parental testing for a variant identified in a child with TBD cannot completely rule out the possibility that a future child will be affected.

After a negative test result, other relatives can be screening with telomere length measurement studies. Telomere length testing may identify other individuals who should follow screening guidelines for TBD findings.

In families where a variant of uncertain significance is identified, additional assessments in family members that include clinical evaluation and telomere length studies can both help identify other relatives with TBD and can help with the

interpretation of the familial variant if other individuals with TBD symptoms are also found to have the familial variant. In the absence of confirmed pathogenicity, relatives should be screened and managed based on their clinical symptoms and telomere length measurements under the guidance of their medical team.

Samples for Telomere and Molecular Genetic Testing

Different samples may be obtained from an individual for testing based on the technology used for testing and/or other indications. Currently, blood is the sample of choice for telomere length testing. Molecular testing of TBD-associated genes can be performed on blood, saliva, buccal (the area of the cheek inside the mouth) scrapings, bone marrow, or skin. If an individual has myelodysplastic syndrome (MDS), leukemia, previously underwent hematopoietic cell transplant, or is receiving certain treatments like transfusions, germline molecular genetic testing from blood, saliva, buccal scraping, or bone marrow samples may not be accurate. In such situations, genetic testing should be performed on a skin biopsy.

In some cases, genetic testing can be complicated by mosaicism. Mosaicism for a specific genetic disorder may occur after conception in one of two ways. An individual may acquire a somatic disease-causing variant that was not in their original germline cells. Additionally they may have a revertant mosaicism in which they originally had a disease-causing variant that was changed back to the "normal" DNA sequence. It is difficult to know when the mosaicism associated variant occurred, so which organ system(s) are involved usually cannot be identified. Suspected revertant mosaicism has been reported in TBD and has the potential to lead to a false negative finding on samples containing blood [62]. If an individual with clinical symptoms highly suggestive of TBD has negative testing, repeat studies on an alternate tissue such as skin can be considered.

Reproductive Options

There are multiple reproductive options available for individuals with an increased chance of having a child with TBD that can be reviewed in detail by a preconception or prenatal genetic counselor. Decisions regarding reproductive planning are personal choices based on individual beliefs, religious practices and ethical values. Families may choose to have an unassisted pregnancy without genetic testing until after the birth of the child and/or wait until the child shows clinical signs of the TBD. Other families may choose not to have children or may consider adoption.

Other individuals may wish to have children with the help of various reproductive technologies. There are currently several options for reducing the chance a child will have the familial TBD including using donor gametes (egg or sperm), prenatal diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) for known variant(s), and in vitro fertilization (IVF) with preimplantation genetic testing (PGT). Prenatal diagnosis is not expected to impact treatment during pregnancy, thus prenatal diagnosis is most commonly sought either for medical management planning following delivery, for consideration of pregnancy termination, or to confirm IVF-PGT results. IVF-PGT can also select for the HLA (human leukocyte antigen) type of an embryo to have a child who is both unaffected with TBD and a potential bone marrow donor to an affected sibling who may need a transplant in the future.

The process of IVF with PGT is time consuming, as well as physically, psychologically, and financially demanding. In order to confirm that the laboratory performing PGT will be able to identify the presence or absence of the variant(s) in an embryo, DNA samples from the individual with TBD and their parents and possibly other family members are requested in order to develop an accurate genetic test. Multiple IVF and PGT cycles may be required to achieve a pregnancy. PGT is not a guarantee that a child will be unaffected but rather a test that reduces the possibility that a future child will have a TBD.

Telomere length measurements cannot be performed as a part of IVF-PGT or prenatal diagnostic testing at this time, thus families who do not have a known familial variant have fewer reproductive options available to them. A blood sample can be obtained after birth for telomere length analysis. For families with a VUS in a TBD-associated gene, our incomplete understanding of the genetic finding limits the availability and utility of reproductive options. Families with uncertain findings should seek genetic counseling to learn more about their options based on the current interpretation of the familial variant. Women with a TBD who are considering pregnancy should be offered consultation with a medical provider to discuss potential health risks during pregnancy (See Chapter 21, Gynecologic and Obstetric Considerations). Surrogacy may be an option for women with a TBD depending on laws and services in their area.

Summary

Genetic testing for TBD requires a comprehensive clinical and family-oriented approach. Genetic counselors should be included as an integral part of the medical team for patients and families with TBD. A local genetic counselor can be found through the https://www.aboutgeneticcounselors.org/ website.

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Chapter 6

Dermatologic Manifestations

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Introduction

Involvement of the skin, nails, and oral mucosa are hallmark features of dyskeratosis congenita (DC). In fact, the original description of DC was based upon the clinical triad of reticulated (net-like) skin pigmentation, fingernail and toenail abnormalities, and oral leukoplakia (whitish plaques inside the mouth). However, not all patients have these findings, which may develop over time (Figure 1) [1]. In addition to the diagnostic triad, individuals with DC and related Telomere Biology Disorders (TBDs) may develop numerous other bothersome cutaneous skin-related complications including hair loss, skin cancer, and excessive sweating (hyperhidrosis). The focus of this chapter is to review the dermatologic manifestations in individuals with DC/TBDs and examine management and treatment strategies.
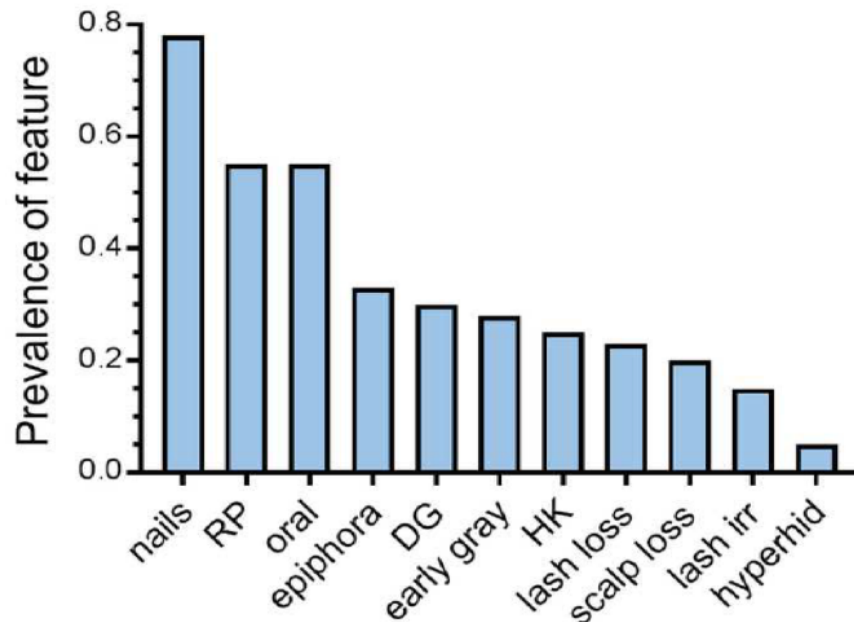
Figure 1. Prevalence of mucocutaneous findings in 60 patients with DC (Adapted from Ward et al, *J Am Acad Dermatol* 2018).
DG, Dermatoglyphic changes; early gray, graying of the hair before the age of 30 years; HK, hyperkeratosis of the palms and/or soles; hyperhid, hyperhidrosis; lash irr, blepharitis or irritation due to eyelashes; lash loss, thinning or sparseness of the eyelashes; nails, nail dystrophy; oral, leukoplakia or oral squamous cell sarcoma; RP, reticulate skin pigmentation; scalp loss, premature hair thinning or balding of the scalp

Skin Cancer

Data from literature reviews and groups (cohorts) of individuals with DC/TBDs have identified an overall increased risk of several cancers for affected individuals [2, 3].

Head and neck squamous cell cancer (HNSCC) and cutaneous squamous cell cancer are the most frequently reported solid organ tumors in this population. Individuals with DC/TBDs tend to develop these malignancies at a younger ages than is typical in the general population [2] In a review of eight individuals with DC/TBDs and cutaneous

squamous cell cancer reported in the literature, the median age at onset was 21 years, significantly earlier than in the general population, with a median age of 68 years old [2].

Squamous cell cancers of the skin generally present as slow-growing pink-to-skin-colored flat growths or raised bumps and may be associated with overlying scales (flaking skin). The most common locations for squamous cell cancers of the skin are areas frequently exposed to sun, including the head and neck, upper trunk, and upper extremities. However, they may occur on any site of the body.

General risk factors for squamous cell cancers include sunlight (ultraviolet light) exposure, radiation, and the chronic use of medications that suppress the immune system (immunosuppressants). Voriconazole, a medication used to treat fungal infections, may also increase the risk of squamous cell cancer [4]. There is also an association between the human papillomavirus (HPV) and squamous cell cancers; however, data suggests individuals with DC/TBDs may develop squamous cell cancers independent of this HPV association [5].

There are multiple surgical (e.g., excision, Mohs micrographic surgery) and non-surgical (e.g., topical chemotherapy, radiation therapy) options to treat skin cancers. The most appropriate treatment depends on factors such as the size and differentiation of the tumor, risk of recurrence, the location on the body, and the anticipated cosmetic appearance following treatment.

Because of the increased risk of skin cancer, prevention strategies are highly recommended for individuals with DC/TBDs. Useful strategies include:

- Regular use of sunscreen or sunblock when outdoors, and use of a daily moisturizing lotion with sunblock
- Wear hats and sun-protective clothing when outdoors to prevent excessive sun exposure
- Limit outdoor time during hours of peak sun exposure (between 10 am and 4pm)

- Be mindful of reflected sun from water and snow when engaging in outdoor activities
- Avoid tanning beds
- Perform regular skin self-examinations to look for new or changing skin growths

In addition, annual full body skin examination by a dermatologist is recommended [6].

Thickening of Palms and Soles

The thickening of the skin on the palms and soles seen in individuals with DC/TBDs may be referred to by the medical term hyperkeratosis. These changes are due to increased thickness of the upper cell layers of the skin overlying these areas (Figure 2).

The mainstay of treatment for hyperkeratosis of the palms and soles is the liberal use of topical moisturizers (emollients) which contain agents that break down keratin, the fundamental component of the excess thickened skin. Agents that may be used for both moisturizing and breakdown of keratin include the following:

- Urea cream
- Salicylic acid cream or compounded ointment
- Cream or lotion containing lactic acid with ammonium hydroxide

Use of a daily moisturizer for maintenance of generally healthy skin on the remainder of the body is also recommended.

Figure 2. Hyperkeratosis: Thickening of the skin of the palm. Image obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

Hair Changes

DC/TBDs is associated with several changes in the appearance and quantity of hair. The hair of individuals with DC/TBDs shows structural abnormalities in both the hair shaft and in the hair cuticle, which may contribute to the thin appearance and early hair loss [7].

Individuals with DC/TBDs often experience graying of the hair at an early age. Additionally, hair loss (alopecia) also tends to occur at an early age in DC/TBDs. This may affect the hair of the scalp, eyebrows, eyelashes, or body. Patients with loss of eyelashes may experience an increase in eye irritation from entry of dust particles or other foreign material and may benefit from protective eyewear. To date, no studies have looked specifically at treatments for hair loss in individuals with DC/TBDs. Minoxidil is an over-the-counter topical treatment that may be used for thinning of scalp hair. Side effects of minoxidil include contact dermatitis and unwanted facial hair growth. Bimatoprost is an ophthalmic solution applied to the base of the upper

eyelashes that causes the lashes to grow longer, fuller and darker. Side effects of bimatoprost include pigmentation of the eyelids and iris which may be permanent.

Skin Pigmentation

One of the diagnostic skin changes associated with DC is the net-like pattern of skin pigmentation termed reticulate pigmentation. The common appearance is of gray-brown skin pigmentation along sites of flexion, including the neck, shoulders, arms, and chest, as seen in Figure 3. An additional finding of skin changes in DC/TBDs is poikiloderma, or thinning of the skin (atrophy) accompanied by the formation of fine blood vessels (telangiectasias) and areas of both increased (hyper-) and decreased (hypo-) pigmentation. While these features may aid in the diagnosis of DC/TBDs, there are no studies examining the impact of treatments specifically aimed at reversing these changes. Liberal use of daily moisturizers can be used to decrease roughness associated with symptomatic dry skin.

Figure 3. Reticulated pigmentation on the neck. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

Skin changes following allogeneic hematopoietic cell transplantation (HCT) or solid organ transplantation for DC/TBDs have not been extensively examined but deserve close medical attention. Individuals who have undergone transplantation and their caregivers should be attuned to new or changing skin lesions for surveillance of skin cancers. Those who underwent HCT should be carefully followed for the development of cutaneous graft versus host disease, which may closely mimic the poikilodermatous skin and nail changes seen in DC/TBDs [6]. Regular visits with a dermatologist are encouraged for skin cancer screening in patients following transplantation.

Hyperhidrosis

Up to 15% of patients with DC/TBDs experience hyperhidrosis, or excessive sweating [8]. Sweat glands are located in highest concentration in the palms, soles, and axillary regions of the body. Excessive sweating can have a significant impact on an individual's quality of life, and several treatment strategies exist for the management of hyperhidrosis.

Treatments for hyperhidrosis include [9]:

- Avoidance of specific triggers which may worsen hyperhidrosis such as alcohol and spicy foods
- Topical aluminum chloride powder, roll-on, or sprays
- Topical glycopyrronium cloths
- Botulinum toxin injections
- Systemic anticholinergic agents such as oxybutynin or glycopyrrolate
- Iontophoresis with topical anticholinergic agents

No studies have been performed to date specifically examining the efficacy of these treatments in individuals with DC/TBDs, but all have been shown to offer some relief of hyperhidrosis in various clinical scenarios.

Adermatoglyphia

Lack of fingerprints – adermatoglyphia – is another potential dermatologic finding of DC/TBDs (Figure 4). In some individuals it is thought to be secondary to a disorder in formation of the epidermis (the top layer of skin) during development as an embryo. In others, it may develop over time due to the hyperkeratosis of the palms [10, 11] Caregivers and individuals with DC/TBDs should be aware of this potential skin complication, as it may impact such processes as immigration and obtaining government identification [11].
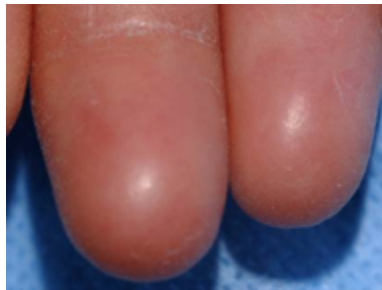
Figure 4. Adermatoglyphia: Loss of fingerprints. Image obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

Nails

Nail involvement in DC/TBDs can range from nail malformation (onychodystrophy) to small nails (micronychia) to complete absence of the nail plate (anonychia). Several nail changes are shown in Figure 5. Common forms of onychodystrophy that occur in DC/TBDs include longitudinal grooving of the nail plate (onychorrhexis) as well as splitting of the nail (onychoschizia).

Figure 5. Nail changes in DC: onychoschizia, longitudinal ridging, and micronychia. Images obtained after informed consent from participants in the Cancer in Inherited Bone Marrow Failure Syndromes Study, ClinicalTrials.gov Identifier: NCT00027274. Courtesy of Neelam Giri, MD and Sharon Savage, MD, National Cancer Institute.

As with the skin findings of DC/TBDs, nail involvement may also mimic features of other conditions. The nail changes have been reported to be similar to those in other cutaneous conditions such as lichen planus and graft versus host disease after HCT.12

There are limited data on management of nail changes specifically in DC/TBDs. Brittle, fragile nails may also impact quality of life by causing repeated injury or discomfort. Keeping the nails trimmed and use of an emery board to dull rough or sharp nail edges may reduce the discomfort of incidental nail injury during day-to-day activities. Additional strategies that may be useful for the treatment of fragile nails include [13]:

- Reduce excessive exposure to water, detergents, and prolonged hand washing
- Avoid long-term use of artificial nails from salons
- Nail polishes and lacquers may help to strengthen brittle nails

- Vitamin supplements such as biotin may provide some strength to brittle nails

Daily Skin Care

As the skin is a clearly visible organ to individuals and to the outside world, an unhealthy or abnormal appearance of the skin can have a potential impact on one's quality of life or self-esteem. Strategies to maintain skin health include:

- Review the daily sun protection strategies outlined above in the skin cancer section
- Use a daily moisturizer, applied daily after showering or bathing, to help maintain the skin's natural water content
- Stay well-hydrated throughout the day with adequate water intake
- Avoid abrasive or overly-drying soaps or detergents
- Perform regular skin self-exams for any new or changing skin lesions

In addition, annual full body skin examination by a dermatologist is recommended [5].

Conclusion

The dermatologic manifestations of DC/TBDs may involve the hair, nails, skin, and mucosal surfaces. Given the increased risk of cutaneous cancers in this population and the clinical overlap with other skin conditions, we recommend regular surveillance by a dermatologist for individuals with DC/TBDs for both diagnostic evaluation and therapeutic management.

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Chapter 7

Ophthalmic Manifestations

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Introduction

Ophthalmic manifestations have been reported in approximately 40% of individuals with dyskeratosis congenita (DC) [1, 2]. Most studies of eye-related complications have been reported in individuals with classic DC, Revesz syndrome, Hoyeraal-Hreidarsson syndrome (HH), or Coats plus. Some of these complications have also been reported in individuals with Telomere Biology Disorders (TBDs), in general, but quantitative data are limited. Ophthalmic complications can be divided into changes affecting the anterior segment and adnexa (eyelids, eye lashes and lacrimal [tear duct] system) and those affecting the retina.

Anterior Segment and Adnexa

Changes to the anterior segment and adnexa include punctal atresia and nasolacrimal duct obstruction, trichiasis (mis-directed eyelashes), loss of eyelashes, entropion (in-turning of the eyelids and eyelashes), ectropion (out-turning of the eyelids and eyelashes), conjunctivitis (infection of the conjunctiva), corneal scarring, corneal ulceration (erosion of the outer surface of the eye) and perforation, and cataracts [1, 3-9].

The most common finding in individuals with TBDs is obliteration of the lacrimal drainage system, which can present with either absent punctae or nasolacrimal duct obstruction. The individual might experience constant tearing, frequent episodes of conjunctivitis, episodes of blepharitis (inflammation of the eyelids), or corneal ulcers. Treatment of tear flow obstruction is surgical, by either dacryocystorhinostomy (DCR), whereby an opening is created between the lacrimal sac and the nasal cavity, or by insertion of Jones tubes, glass implants placed to allow direct drainage from the conjunctival fornix into the nose.
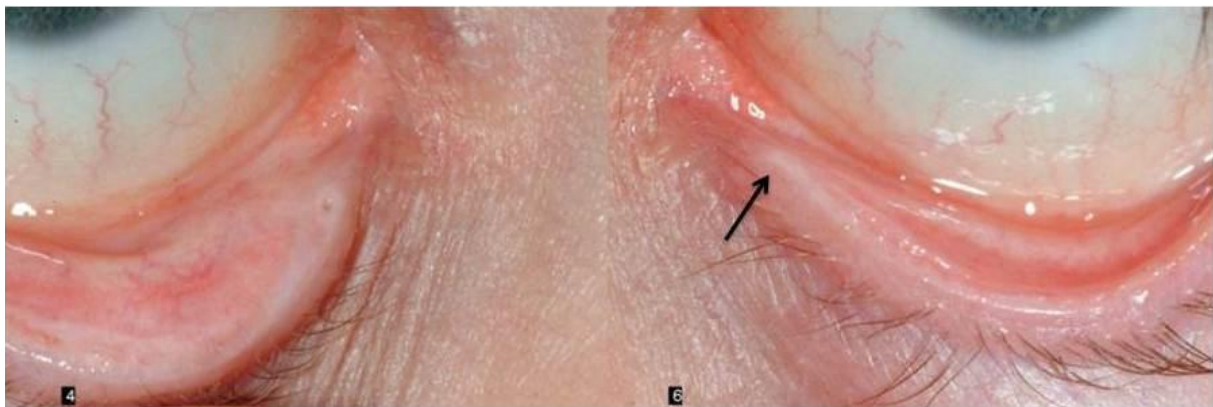
Figure 1. Photograph of a patient with dyskeratosis congenita demonstrating an absent punctum in the left eye (arrow).

Entropion, ectropion, and trichiasis may be secondary to epithelial abnormalities of the skin and mucous membranes of the eye, and can lead to recurrent blepharitis,

conjunctivitis, keratitis, corneal scarring, and eventually to decreased vision if untreated. Entropion and ectropion can be surgically repaired, and misdirected eyelashes seen in trichiasis can be either temporarily or permanently removed. Early recognition of these potentially significant complications of TBDs leads to optimal management and outcomes.
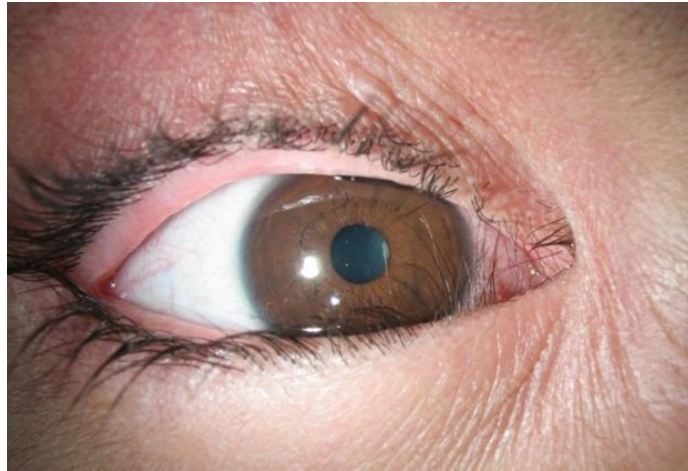
Figure 2. Entropion and trichiasis in an individual with dyskeratosis congenita.

Posterior Segment

Optic nerve atrophy [3, 10, 11] and retinal vascular changes have been described in patients with DC/TBDs. Retinal changes can include hemorrhages, areas of non-perfusion, retinal neovascularization, exudative retinopathy, and retinal detachment [2, 9, 10, 12-20] These changes require early recognition and management to avoid the potentially devastating complication of vision loss if untreated. Fluorescein angiography, ideally widefield, is indicated if any significant vascular abnormalities are identified during the retinal exam of a patient with DC/TBD. Treatment may include laser photocoagulation, or vitreoretinal surgery in advanced cases.

Two clinical variants of TBDs are associated with a greater risk of retinal abnormalities. Individuals with Revesz Syndrome develop exudative retinopathy, in addition to aplastic

anemia and central nervous system abnormalities [17-19]. Individuals with HH can also display an increased frequency of retinal neovascularization. However, the prevalence of retinopathy is likely underappreciated. Widefield fluorescein angiography on individuals with DC/TBDs has shown that the vast majority of individuals will likely harbor retinal vascular alterations [21].
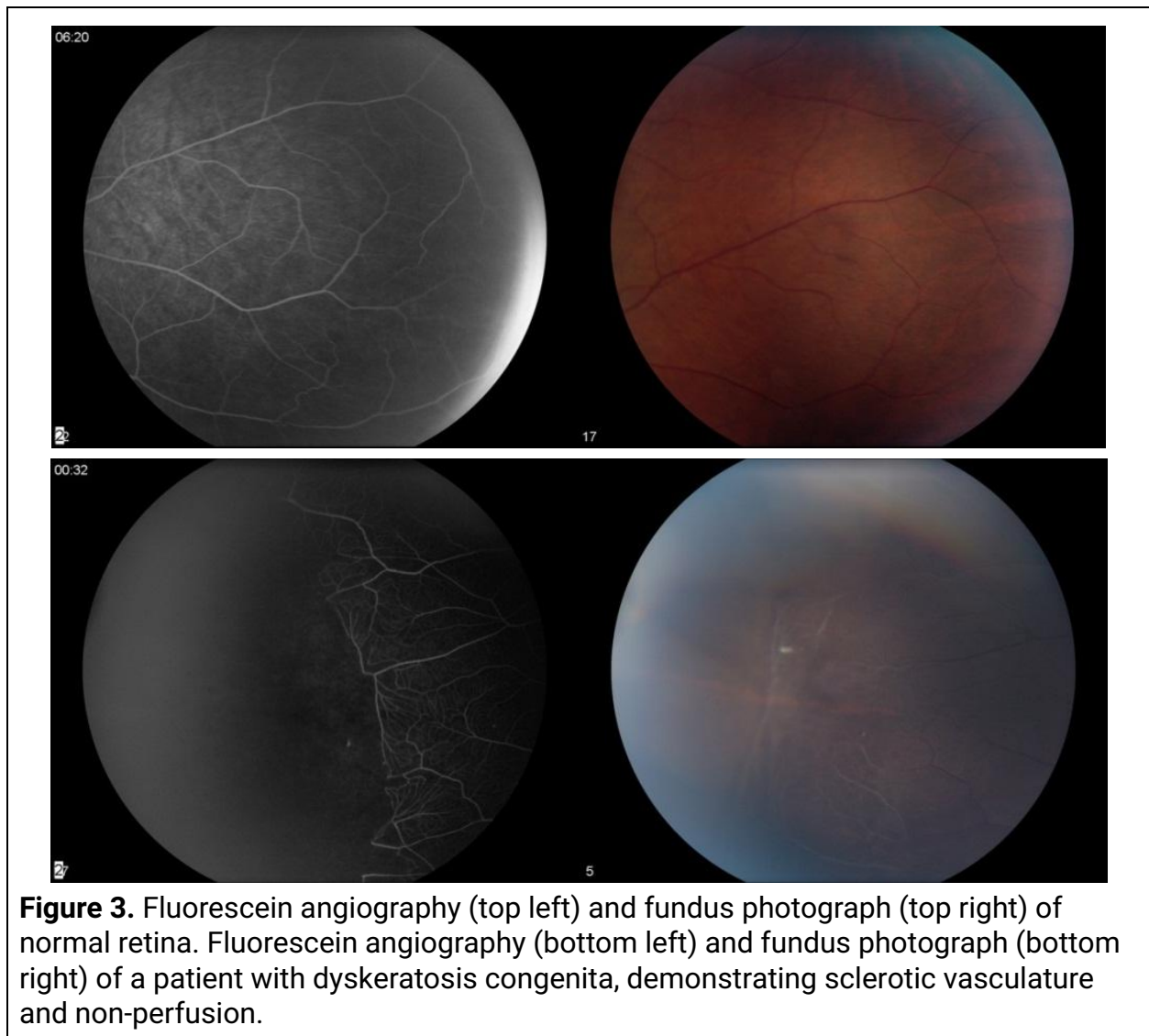
Figure 3. Fluorescein angiography (top left) and fundus photograph (top right) of normal retina. Fluorescein angiography (bottom left) and fundus photograph (bottom right) of a patient with dyskeratosis congenita, demonstrating sclerotic vasculature and non-perfusion.

Additional Complications

In addition to the syndrome-specific manifestations of TBDs, side effects of treatment required for other TBD-associated manifestations can result in other ocular complications. Therapeutic radiation can cause radiation retinopathy, and systemic corticosteroid therapy associated with hematopoietic cell transplantation (HCT) may cause glaucoma or cataract. Patients undergoing HCT require a detailed eye examination and follow-up monitoring to identify potential problems.

Recommendations

A baseline ophthalmic evaluation should be part of the initial evaluation of every patient with TBDs, and subsequent periodic examinations should be part of routine care.

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Chapter 8

Dental and Oral Complications

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Introduction

The oral phenotype of dyskeratosis congenita (DC) and related Telomere Biology Disorders (TBDs) is characterized by leukoplakia and developmental anomalies of the permanent teeth, such as decreased root/crown ratio and mild taurodontism (vertically enlarged pulp chamber and shortened roots) [1]. Individuals undergoing hematopoietic cell transplantation (HCT) at an early age are susceptible to disruptions in tooth development, as well as development of chronic oral graft versus host disease (GVHD), reduced salivary flow (xerostomia), and thrush (oral candidiasis), each of which require medical management.

Oral Leukoplakia

Oral leukoplakia associated with DC/TBDs manifests clinically as a heterogeneous mucosal lesion and may develop at any age, from childhood to later in adult life. Often localized to the dorsum (top) of the tongue, buccal mucosa (insides of the cheeks), palate (roof of the mouth) and/or gingiva (gums), the lesion(s) can present as a fine reticular or a plaque-like white area, with or without peripheral erythema (redness). The clinical presentation varies among individuals, with oral signs and symptoms developing at different rates. Very little is known about the clinical or histopathological features of oral leukoplakia in DC/TBDs; however, they are thought to contribute to an increased risk of developing head and neck squamous cell carcinoma (HNSCC).

Head and Neck Squamous Cell Carcinoma

Approximately 660,000 cases of HNSCC will arise globally each year, 54,000 of which will be in people in the United States [2, 3]. HNSCC risk factors in the general population include exposure to carcinogens, most notably tobacco smoking and alcohol consumption, infection with high-risk types of human papillomavirus (HPV), and genetic predisposition, such as in DC/TBDs.

HNSCC is considered a heterogeneous disease both at the molecular and clinical levels, with the existence of at least two genetic subclasses: HPV-positive and HPV-negative tumors [4]. Despite advances in diagnosis and treatment, the five-year survival rate for HNSCC continues to be approximately 50% [5, 6]. Most individuals who develop HNSCC had a clinically visible premalignant oral lesion (dysplasia) prior to developing cancer; early diagnosis and surgical management of oral dysplasia/HNSCC is extremely important in reducing patient complications.

Oral leukoplakia itself is not uncommon in the general population, with an estimated prevalence from less than 1% to more than 5% [7-10]. The rate of its malignant

transformation into HNSCC varies from near nil to about 20% over one to thirty years [11-13].

Individuals with DC/TBDs have a very high risk of developing cancer. Specifically, the ratio of observed to expected (O/E) cancers was 4-fold greater in DC/TBDs when compared with the general population (see Chapter 9, Solid Tumors). Forty percent of the most common solid tumors in DC/TBDs were found to be HNSCC, including an approximately 216-fold increase in the O/E ratio for cancer of the tongue [14]. While bone marrow failure continues to be the main cause of mortality in DC/TBDs, these numbers suggest an independent high risk of mortality arising from HNSCC.

It has been suggested that non-homogeneous oral leukoplakia carries a higher degree of malignant transformation risk compared with the homogeneous variants; however, there is no reliable method to identify which oral lesion will transform into cancer and which will not [15]. Clinical [16], histological [17], and molecular markers [18, 19] may aid in assessing the risk of an individual patient to develop cancer; however, currently there is no evidence-based and clinically useful predictor of malignant transformation in people with DC/TBDs or in the general population.

Oral Lichen Planus

Lichen planus (LP) is an autoimmune T cell-mediated mucocutaneous inflammatory disease of unknown etiology [23]. Occurring in approximately 1% of the general population and most frequently in women 30 to 60 years of age [24], LP can affect the oral mucosa, genitals, and skin. The clinical presentation of oral lichen planus (OLP) and the oral lesions associated with DC/TBDs is phenotypically identical.

As with oral leukoplakia in DC/TBDs, OLP has variable clinical characteristics and presentations. The most common type (reticular) is characterized by lacy white striae located bilaterally on the buccal mucosa, gingival, or tongue. The erosive (red or ulcerated), atrophic, and plaque-like versions of OLP are thought to have greater

malignant potential than the reticular type, perhaps because of chronic inflammation. Reticular OLP is often asymptomatic, but the atrophic and ulcerative forms can cause symptoms ranging from a burning sensation to severe, unremitting oral pain [25, 26].

The World Health Organization (WHO) classifies OLP as a precancerous disease in the general population. However, this designation is controversial. The frequency of malignant transformation in the general population has been found to range anywhere from 0.4% to more than 6% [8, 9]. 1-5% of OLP lesions will undergo malignant change to squamous cell carcinoma (SCC) of the mouth [27-30]. Between 1 and 3% of vulvar lichen planus lesions in the general population may develop into SCC [31, 32] while a small but unknown percentage of penile lesions will transform [33, 34]. Chronic inflammation appears to play an important role in the promotion of malignant transformation of oral mucosa in some disorders. Both OLP with dysplasia and oral lesions of individuals with DC/TBDs show evidence of chronic inflammation coincident with a higher than normal rate of transformation to HNSCC. Therefore, OLP with dysplasia may serve as a unique disease model for studying the high rate of oral malignancy associated with DC/TBDs.

NCI Cohort Study

The National Cancer Institute (NCI) cohort study of inherited bone marrow failure syndromes (02-C-0052) evaluated 44 individuals with DC/TBDs between September 2003 and June 2012, and included detailed oral examinations, radiographs and clinical photos. The overall prevalence of oral leukoplakia was 64%: 75% in children and 50% in adults, with the youngest patient being 3 years old and the oldest 53. 93% of oral leukoplakia was localized to the dorsal tongue (plaque-like and reticular lichenoid white lesions with papillary atrophy). Of those with tongue lesions, 20 out of 26 (77%) had papillary atrophy. Only seven had oral ulcerations at the time of examination, and very few had accompanying erythema. The presence or absence of oral symptoms with DC/TBD-associated oral lesions is not known but may be underreported.

Variants in five DC/TBD-related genes were identified among 32 of the 44 individuals evaluated in this study: *WRAP53*, *DKC1*, *TERC*, *TINF2*, and *TERT*. The remaining patients did not have a known genetic cause of disease. Ninety percent (9/10) of patients with *DKC1* variants but only 17% (1/6) of those with *TERT* variants had oral lesions, which suggests an association between development of oral leukoplakia and the specific DC/TBD gene variant (Table 1).

Table 1. Presence of oral lesions by gene in patients with DC/TBDs (unpublished data).

Gene Mutation	n	Oral Lesions	No Oral Lesions	p value	RR	95% CI
TERT	6	1 (17%)	5 (83%)			
DKC1	10	9 (90%)	1 (10%)	<0.0001*	0.17	0.11-0.28
TERC	3	2 (67%)	1 (33%)	<0.0001*	0.28	0.18-0.44
TINF2	11	6 (55%)	5 (45%)	<0.0001*	0.36	0.24-0.56
Unknown	12	8 (67%)	4 (33%)	<0.0001*	0.28	0.18-0.43
WRAP53	2	2 (100%	0	<0.0001*	0.14	0.09-0.23

Eight subjects evaluated in the NCI cohort have had multiple oral biopsies with histopathological results that ranged from benign, chronic inflammation, pyogenic granuloma, and hyperkeratosis to moderate dysplasia and HNSCC.

Clinical Implications: Patients

Oral leukoplakia in DC/TBDs develops at an unusually young age and is characteristic in appearance and location. Since most leukoplakias are asymptomatic, the need for treatment is primarily based on the precancerous nature of the lesion, and this can only be determined by tissue biopsy. A region of the oral lesion may transform into oral cancer, but it is not currently known what that progression looks like in DC/TBDs. Early

diagnosis and surgical management of oral dysplasia and HNSCC is extremely important in reducing morbidity.

Therefore, it is recommended that a dentist or dental specialist (oral medicine or oral and maxillofacial surgeon) screen individuals with DC/TBDs for oral lesions every six months, in addition to an otolaryngologist (ears, nose, and throat doctor, ENT) evaluation every six to 12 months. The frequency of follow-up may increase to visualization of oral lesions every 2 months if it is clinically indicated.

Fiber optic examinations are recommended from the age of 10 years to visualize the posterior oropharynx. The frequency of follow-up may be increased in the presence of histologically confirmed oral dysplasia, or a history of oral cancer. Clinical photos are recommended longitudinally to follow the progression of oral lesions. Persistent oral lesions should be biopsied when clinically indicated. Surgical removal of oral lesions in the absence of a precancerous histological diagnosis is not recommended since it may compromise surveillance efforts.

Oral leukoplakia associated with DC/TBDs is similar in appearance to oral lichen planus. When symptomatic, it may present as ulcerations on the tongue or buccal mucosa that do not resolve within two weeks or recur over time.

Topical steroids used to manage ulcerative oral lichen planus may be helpful in reducing the size and duration of the oral ulcerations in DC/TBDs, and may include fluocinonide, dexamethasone rinse 0.5mg/5mL, and clobetasol cream or ointment (0.05%). Frequency of application should be determined by the prescriber. Associated pain is managed primarily via topical anesthetics. It is not known whether treatment of symptomatic oral leukoplakia in DC/TBDs will alter the risk of malignant transformation.

Clinical Implications: Clinicians

In relation to lesions seen in the general population, oral leukoplakia of DC/TBDs tends to occur in patients younger than 50 years of age and in the absence of risk factors, like

smoking or alcohol use. Leukoplakia may be the first manifestation of DC/TBDs and can be easily identified during routine dental and medical examinations.

The gold standard for oral cancer diagnosis remains tissue biopsy with histological assessment; however, there are adjunctive clinical diagnostic tools that aid in early detection, including toluidine blue dye (TB) and fluorescent visualization imaging systems.

Toluidine Blue

TB is a member of the thiazine group of metachromatic dyes. These dyes bind to DNA and are partially soluble both in water and alcohol. Theoretically, dysplastic and malignant cells have higher nucleic acid content than normal, and thus staining of suspicious lesions with this dye can aid recognition of mucosal changes. Used since the early 1980s, lesions that take up the TB dye are six times more likely to become oral cancers [35]. The TB test appears to be highly sensitive (97.8%– 93.5%) but less specific (73.3% - 92.9%), mainly because of high false positive results [36, 37]. TB has been shown in single- and multicenter studies to be useful in identifying OLPs and OSCC and can provide information about lesion margins and so aid in biopsy site selection [38-42].

Recently, molecular studies on TB stained lesions demonstrated a link between carcinoma and loss of heterozygosity (LOH). This concept refers to deletion of regions of chromosomes (for example 3p, 17p, and 9p) known to be frequently lost in head and neck cancers and dysplasia. LOH occurs early in oral carcinogenesis, but the patterns of such loss can be predictive of the risk of progression. Oral lesions that stained positive with the TB dye were much more likely to have LOH [35, 43]. The loss of heterozygosity has not been evaluated in dysplastic DC/TBD lesions.

Tissue Autofluorescence

The value of tissue autofluorescence in the screening and diagnosis of precancerous lesions of the lung, uterus, cervix, and skin is well documented. In the past decade, several forms of autofluorescence technology have been developed to inspect the oral mucosa, including VELscope R (LEDDental, Inc., of White Rock, British Columbia, Canada). This U.S. Food and Drug Administration- and Health Canada-approved technology uses blue/violet light (400-460nm) to illuminate oral tissue. By visualizing these oral tissues through a light filter, normal tissue appears pale green, while abnormal tissue has a "loss of fluorescence" and appears dark brown or black. Like TB, autofluorescence may aid in choosing tissue biopsy sites, as well as visualizing surgical margins [44, 45].

There is general consensus that the clinical stage at time of diagnosis of HNSCC is the most important predictor of recurrence and mortality in oral cancer patients. The time to diagnosis is influenced by multiple clinical and sociodemographic variables, including patient reluctance to consult a health-care professional due to lack of access to health care, especially in patients with low socioeconomic status.

Clinicians can improve patient survival rate if a cancerous lesion is detected at an early stage, or if a precursor lesion (dysplasia) is discovered and treated prior to malignant progression. To this end, studies have shown that health-care providers would benefit from system-wide educational updates on oral cancer prevention and early detection.

Treatment of HNSCC

Treatment options are limited to the surgical removal of regions of oral dysplasia (moderate and severe), carcinoma *in situ*, and HNSCC. The extent of surgical resection depends upon the size and location of the tumor, so early detection is paramount to reduce morbidity and mortality. Laser ablation of regions of lichenoid striae in the absence of dysplasia is not recommended as the lesions are likely to recur. In addition, surgical alteration of normal oral architecture may compromise surveillance efforts.

Dental Manifestations

In addition to oral leukoplakia, DC/TBD patients may exhibit alterations in tooth development. Short dental roots resulting in unfavorable root/crown ratios may affect dental restorative considerations. Root/crown ratios are a non-specific finding and normally vary between different races, genders and arch (maxillary versus mandibular teeth) [46]. Short roots may complicate treatment planning in orthodontics and must be considered in prosthodontic anchorage and estimating the ability of a tooth to carry masticatory forces. In Atkinson et al's study of 17 individuals with DC/TBDs, a decreased root/crown ratio was found in 75% of patients with sufficient tooth development to permit evaluation [1].

Taurodontism affects the molars and is characterized by vertical enlargement of the pulp chamber and reduced root size. It is a frequent finding in early humans and is found in some developmental syndromes. While the exposed portion of the teeth may look overtly normal without any characteristic anomaly on visual inspection, the floor of the pulp and furcation of the tooth may be shifted apically toward the root.

This occurs from failure or late invagination of Hertwig's epithelial root sheath, which is responsible for root formation and shaping.

Mild taurodontism was reported in Atkinson et al's study (57% with radiographs and sufficient tooth development to permit evaluation) [1]. The clinical implication of taurodontism is increased risk of pulp exposure because of decay and dental procedures, endodontic treatment challenges, and with the shorter roots, prosthodontic and orthodontic issues as mentioned above [47].

Other oral findings, such as aggressive periodontal disease, hypodontia, increased dental decay, and thin enamel, have been reported, but do not appear to be more common in DC/TBDs than in the general population.

Post Hematopoietic Cell Transplantation Oral Manifestations

Individuals with DC/TBDs who undergo HCT may develop chronic conditions such as oral GVHD, reduced salivary flow (xerostomia), and thrush (oral candidiasis), which may require medical management. Those transplanted at an early age (<10 years) are more likely to experience disruptions in permanent tooth development. To a significant degree, the oral problems associated with HCT can be prevented or minimized with careful management. Consultation with a dental team experienced in caring for patients undergoing HCT procedures should be completed before the start of therapy.

Oral Graft Versus Host Disease

Signs and symptoms of oral GVHD are similar to autoimmune disorders like LP and may present as sensitivity of the lining of the mouth to acidic foods and mint flavored toothpaste. There may also be mucosal ulcerations, erythema and lichenoid striae. Treatment of oral GVHD is only recommended when symptomatic and can often be managed with topical steroid rinses or creams, or systemic immunosuppression. GVHD can also involve the salivary glands, resulting in xerostomia.

Xerostomia

Saliva serves many critical functions in the homeostasis of the oral ecosystem, in the oropharynx and larynx, and in speech and swallowing functions. Diminished saliva either from medications or oral GVHD results in an increased risk of dental demineralization and decay and oral infections such as candidiasis. A chronically dry mouth can also lead to an altered sense of taste, difficulty speaking, halitosis, oral pain, and difficulty chewing and swallowing, culminating in decreased quality of life. Without saliva, teeth do not remineralize, and there is gradual softening of the tooth matrix. The

teeth bend and dentin may fracture and cavitate, and the coronal structure fractures from the supporting root.

In patients with residual salivary gland function, sugarless gum or lozenges may stimulate salivary secretion. Sugar-free popsicles, plain ice cubes, or ice water may be used to keep the mouth moist. Systemic sialogogues may increase production of natural saliva from functional glands. Medications that may be beneficial in stimulating salivary glands include pilocarpine (Salagen), cevimeline (Evoxac), anethole trithione (Sialor), and bethanechol (Urecholine). Saliva replacements like Oral Balance Gel may offer some relief. There is no ideal substitute for saliva when salivary glands are nonfunctional.

For the prevention of rampant dental demineralization and caries in a xerostomic mouth, patients should apply a 1.1% neutral sodium fluoride gel daily (for at least 5 minutes), using a custom-fitted vinyl tray if possible. This practice should be continued daily as long as the mouth remains dry because of low salivary flow rates. High-potency fluoride brush-on gels and toothpastes may be considered in those who are unable or unwilling to comply with the use of fluoride trays.

To prevent dental damage associated with a lack of saliva, patients with xerostomia should increase oral hygiene efforts and avoid foods and medications (like Nystatin Rinse, Mycelex Oral Troches) with high sugar content.

Thrush/Oral Candidiasis

Candida albicans is a yeast-like fungus naturally present in the oral cavity. It may overgrow in xerostomia, when there is disruption of oral bacteria (as caused by systemic antibiotics), or with the use of topical steroids. Infection presents as creamy white plaques on mucosal surfaces and can be wiped off.

Individuals may experience an increase in sensitivity localized to the lining of the mouth. Oral candidiasis may also present as cracking and redness at the angles of the mouth (angular cheilitis).

Topical anti-yeast medications such as nystatin rinse and clotrimazole (Mycelex) oral troches have a high sugar content and should generally be avoided in patients with xerostomia since they can promote tooth decay. Patients using these medications should be warned of the risk and increase oral hygiene efforts accordingly. Amphotericin B (available as a topical suspension [100mg/mL] 1mL up to 4 times daily) and fluconazole are effective systemic antifungals.

Tooth Development

Dental abnormalities have been reported among survivors of childhood cancer. These include dental hypoplasia or agenesis, root stunting, and enamel hypoplasia. The severity of the problem is dependent upon the timing of chemotherapy and radiation, with the greatest impact occurring when treatment is between the ages of three and five [48, 49].

Clinical Management

Helping patients understand how cavities develop assists in preventing them.

Having DC/TBDs does not confer genetic susceptibility to developing cavities (caries); rather, dental decay is a multifactorial problem. Diet, oral bacteria (which form plaque), a decrease in the quality and quantity of saliva, and other factors are implicated. It has been shown that dental decay will not develop in the absence of fermentable dietary carbohydrates [50]. All dietary carbohydrates are cariogenic (dental decay causing) to some degree, and this is influenced not only by the composition of carbohydrate-containing foods but by the sequence and frequency with which they are consumed. Sucrose appears to have the greatest cariogenic potential, and its frequency

of intake is more important than the total amount consumed. Further, solid forms of the sugar are more easily retained on teeth and appear to be more cariogenic than liquid forms.

The lowering of salivary pH (acidification) with the consumption of fruit juices, citric acid in soft drinks, canned pears and apples, for example, can also accelerate demineralization of tooth surfaces [51]. Certain foods appear to be protective. Cheddar cheese, regular milk consumption, and salted peanuts increase oral alkalinity. Cocoa contains substances that inhibit oral acidification. Starchy, fibrous foods require more chewing, and may inhibit the development of dental decay by stimulating saliva and maintaining a more neutral plaque pH. Polyols (sugar alcohols, including the 6- carbon sorbitol and 5-carbon xylitol) are noncariogenic and possibly even anticariogenic, since xylitol is not metabolized by oral bacteria [52, 53].

The composition and rate of saliva flow can impact development of dental decay in several ways. Saliva can act as a buffer, neutralizing bacterial acid byproducts found on tooth surfaces and in carious lesions. The high concentrations of calcium and phosphorus and the low level of fluoride found in saliva may facilitate remineralization of early carious lesions and form caries-resistant surface enamel [54]. Saliva also contains several potentially bacteriostatic agents, including lysozyme, lactoferrin, and secretory immunoglobulins, which may inhibit the metabolism and growth of cariogenic bacteria [55-57].

Reduction in salivary quantity may be a side effect of various medications, including antidepressants, anti-anxiety medications, and antihistamines, or may be a component of post-transplant oral GVHD. Having a dry mouth can accelerate the rate of dental decay and significantly increased oral hygiene efforts are required. Patients should work with their general dentist and hygienist for strategies to prevent dental decay.

Fluoride is the most effective dietary component to exhibit a protective effect against root and surface caries. The mechanism by which it does so is not well understood but is thought to be related to its presence in enamel and dentin, and presumed role in

promoting remineralization of early demineralized areas of the tooth [58]. By exerting an antimicrobial effect, it suppresses cavity-causing oral bacteria [59].

General hygiene recommendations include brushing teeth two to three times a day with fluoridated toothpaste and flossing once a day at a minimum to help prevent tooth decay. Some dentists recommend using prescription strength fluoride toothpaste or antibacterial mouth rinse to aid in reducing oral disease. Biannual dental checkups and cleanings are recommended to monitor for the presence of oral pathology and prevent the development of significant dental decay and gum disease. Precautions during routine dental treatment may be necessary in the presence of low platelet counts and white blood cell levels.

Access to quality dental care for medically compromised patients may be challenging outside of large urban centers. Regional dental schools offer clinics with multidisciplinary dental specialty practitioners and may provide economical dental treatment or be able to recommend a community dentist.

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Chapter 9

Solid Tumors

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Introduction

Cancer has been reported in people with dyskeratosis congenita (DC) and the related telomere biology disorders (TBDs) since DC was first described in the early 20th century. Three of the first reported cases developed oral or skin squamous cell carcinoma (SCC) [1-4]. Long term, prospective studies have aided in quantifying the types of cancer and age at onset in DC/TBDs. There is also a growing understanding of the role of telomeres in cancer biology. This chapter focuses on solid cancers in DC/TBDs. See Chapter 12, Myelodysplastic Syndromes and Acute Myeloid Leukemia in Telomere Biology Disorders for information on blood cancers in DC/TBDs.

Telomeres and Cancer

Telomeres play a significant role in chromosomal stability and telomere dysfunction is implicated in cancer biology [5, 6] (Figure 1). Telomeres consist of long tandem $(TTAGGG)_n$ DNA repeats and a protein structure at chromosome ends that protect genetic information by maintaining chromosomal stability during cellular division. Each time a cell divides, small amounts of telomeric DNA are lost due to DNA polymerase's inability to fully replicate 3' DNA ends. Consequently, telomeres shorten with each cell division and can be a marker of cellular aging. Telomere attrition over time results in a critically short telomere lengths, which triggers cell senescence and/or cell death. Cancer cells can bypass this process through upregulation of certain genes, including *TERT*, *TP53*, and/or *RB6*. This allows cancer cells to overcome short telomere-induced senescence and continue to divide.
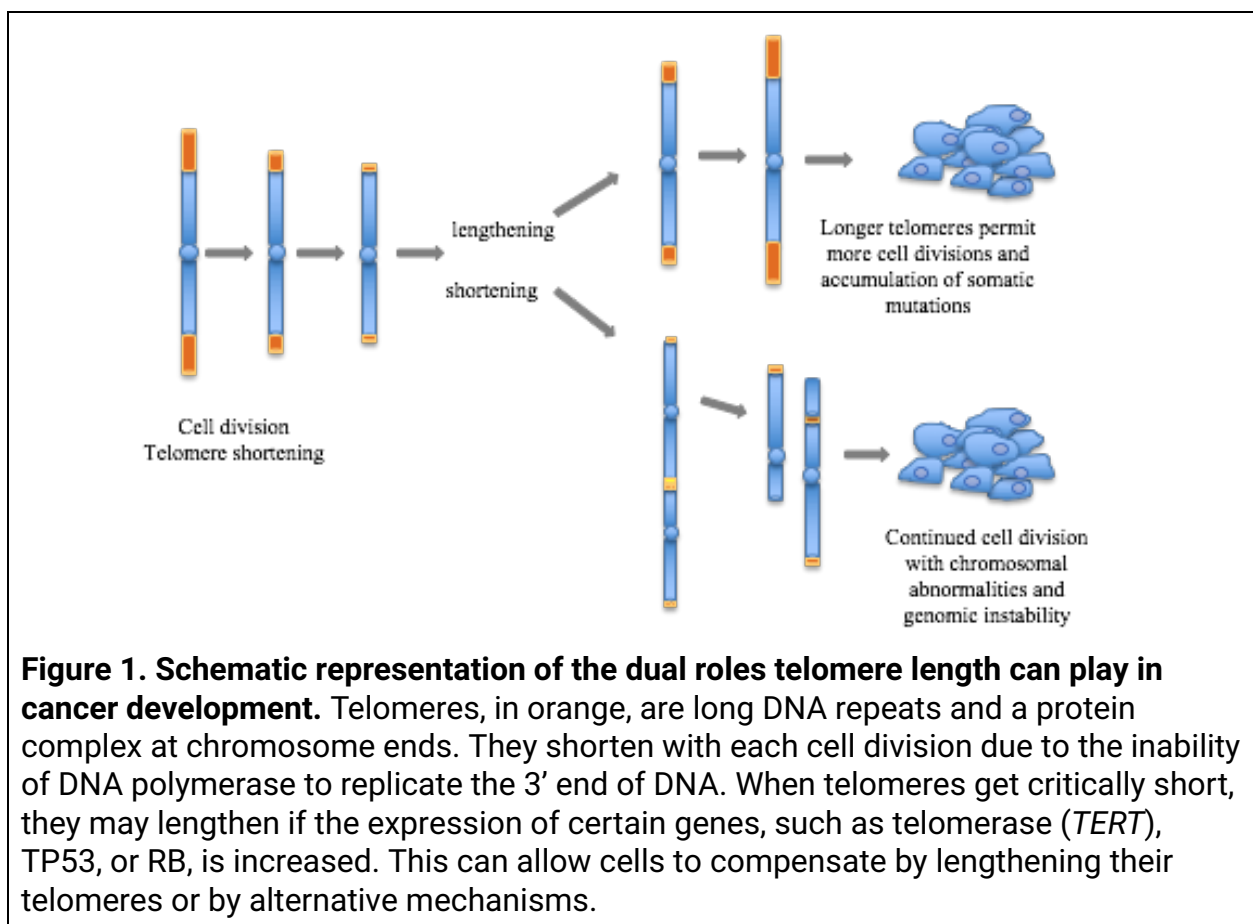
Figure 1. Schematic representation of the dual roles telomere length can play in cancer development. Telomeres, in orange, are long DNA repeats and a protein complex at chromosome ends. They shorten with each cell division due to the inability of DNA polymerase to replicate the 3' end of DNA. When telomeres get critically short, they may lengthen if the expression of certain genes, such as telomerase (*TERT*), TP53, or RB, is increased. This can allow cells to compensate by lengthening their telomeres or by alternative mechanisms.
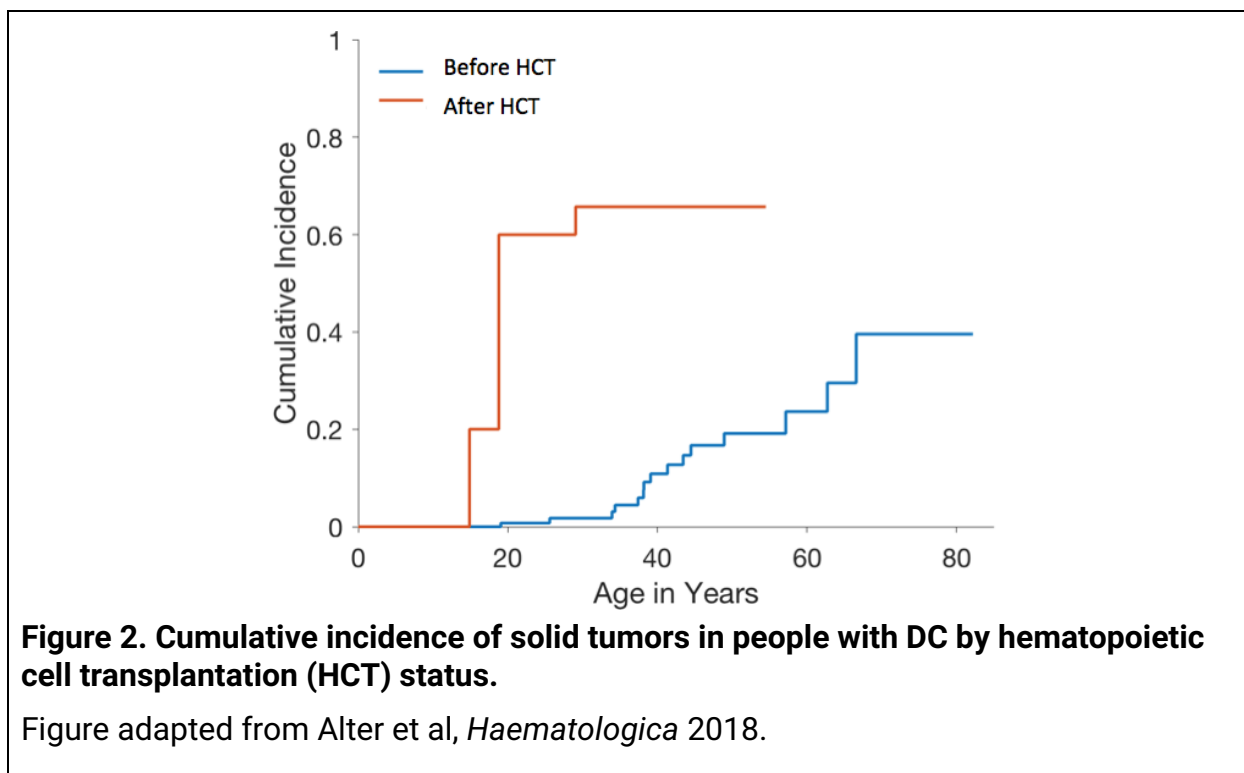
Frequency and Types of Cancer in DC and the Related TBDs

In 2006, the crude rate (number of patients with DC and cancer divided by the total number of DC patients evaluated) of cancer in DC was estimated at around 10% [7]. A subsequent review of the scientific literature published between 1910 and 2008 found reports of cancer in 52 of 552 patients, a similar crude rate of 9.4% [8]. The types of cancer in that report included twenty-five patients with head and neck squamous cell carcinoma (HNSCC, 27 occurrences), eight with stomach, seven with anorectal, and seven with skin cancers [8].

These first studies were informative but not entirely accurate estimates of cancer in people with DC. They did not consider that cases with cancer are more likely to be reported than those without cancer (i.e., reporting bias). Simply comparing percentages of patients with cancer results in a significant underestimate of risk because age, sex, birth cohort, competing risks of adverse events (i.e., pulmonary fibrosis, liver cirrhosis, or hepatopulmonary syndrome) are not considered. Importantly, they also did not provide risk comparisons with the general population. Long-term cohort studies of people with DC/TBDs enrolled regardless of their prior medical history have been helpful in fine-tuning cancer risk estimates, in guiding patient management, and identifying areas in need of further study. However, these studies remain limited because of the wide spectrum of disease manifestations and multiple modes of inheritance in DC/TBDs.

In 2018, investigators at the National Cancer Institute published an updated study on cancer in 197 individuals with DC from 108 families [9]. There were up to 15 years of follow-up from study participants, which equated to 5655 person-years of data. The median age for developing any solid tumor was 38 years (range 18-61). Twenty cancers occurred before hematopoietic cell transplantation (HCT) and three occurred after HCT. Nearly 20% of patients with DC developed at least one solid cancer by age 65 years

(cumulative incidence). Solid cancer was more frequent and occurred at younger ages in patients after HCT (Figure 2).

Figure 2. Cumulative incidence of solid tumors in people with DC by hematopoietic cell transplantation (HCT) status.

Figure adapted from Alter et al, *Haematologica* 2018.

After accounting for age, sex, race, and birth year, the number of observed (O) cancer cases in inidividuals with DC with the expected (E) number of cancer cases in the general population, the data show that those with DC had a 4.2-fold increased risk (O/E ratio) of any cancer (solid organ and blood cancers combined) compared with the general population (Table 1) [9]. Patients who had undergone HCT had an approximately 30-times higher risk of cancer than those in the general population. MDS and AML appeared at a 578- and 24-fold increased risk. The O/E ratio for any HNSCC was 74, the predominant subtype being tongue HNSCC with an O/E ratio of 216.

Table 1. Types of cancer in people with DC and comparison with the general population. Table adapted from Alter et al, *Haematologica* 2018[6]. One hundred ninety-seven patients with DC from 108 families were evaluated.
*Tongue cancer is a subset of head and neck squamous cell carcinoma (HNSCC).
O/E: number of cancers observed in patients with DC divided by the number of cancers expected in someone of the same age in the general population using the NCI Surveillance and End Results database (https://seer.cancer.gov)
Abbreviations: CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma.

	no HCT 197 people with DC 5644 person-years evaluated				After HCT 60 people with DC 248 person-years evaluated			
	Age at cancer	# cases	O/E	95% CI	Age at cancer	# cases	O/E	95% CI
All cancers	38 (18-63)	27	4.2	2.8-6.1	19 (15-29)	3	30	6-8
All solid cancers	38 (18-61)	17	3.1	1.8-4.9	18.8	1	13	0.3-73
HNSCC	38 (18-61)	11	74	37-133	18.8	1	432	11-2404
Tongue*	33 (18-42)	8	216	94-427	18.8	1	1561	40-8699
Blood and lymph node cancers								
AML	40 (28-56)	5	73	23-169				
MDS	31 (4-73)	18	578	343-914				
NHL	57 (43-65)	3	11	2.2-30	29.1	1	141	4-786
Hodgkin lymphoma					14.9	1	164	4-914

Another study evaluated cancer in 180 patients with telomere biology disorders from 113 families, of whom 14 had classic DC (defined as having at least one of oral leukoplakia, nail dystrophy, and/or abnormal skin pigmentation) [10]. MDS and AML were the most common malignancies in this study, occurring in 18 patients (see Chapter 12, Myelodysplastic Syndromes and Acute Myeloid Leukemia in Telomere Biology Disorders for more information). There were five patients with six solid organ cancers (three HNSCC, two anal SCC, and one rectal adenocarcinoma) with a median age at cancer of 35 years (range 25-53). Four of these five patients met the investigators' criteria of classic DC and these four also had pathogenic variants in *DKC1*. This publication and a review from the same group implied that "cancer is relatively rare in short telomere syndromes" [10, 11]. It is true that cancer in DC/TBDs may occur at a lower rate than in other inherited cancer susceptibility syndromes. For example, approximately 90% of people with Li-Fraumeni syndrome due to germline *TP53* pathogenic variants will have at least one cancer prior to 70 years of age [12, 13]. However, cancer in people with DC/TBDs occurs at much higher frequency and much younger ages than in people in the general population [14]. This information is crucial in tailoring cancer screening and prevention recommendations.

Treatment of Cancer in DC/TBDs

Treatment of cancer in people with DC is especially challenging because patients may also have bone marrow failure, pulmonary fibrosis, liver cirrhosis, and/or many of the other clinical manifestations associated with DC/TBDs. Consequently, patients with DC and cancer may have more treatment-related complications than people without DC.

We reviewed the cancer treatment records of 38 patients with DC participating in the NCI IBMFS cohort study (ClinicalTrials.gov Identifier: NCT00027274, https://marrowfailure.cancer.gov) [9] (Vasta et al, unpublished data). Forty-five cancers (before and after HCT) occurred in these 33 patients (non-melanoma skin cancers were

excluded from this analysis). Affected genes were six *DKC1*, one *RTEL1*, nine *TERC*, six *TERT*, six *TINF2*, one *WRAP53*, and nine unknown.

Six patients with AML (median age at AML 36 years, range 25-56) passed away due to therapy-related complications. Two patients had chemotherapy-alone toxicities including infections and acute respiratory distress syndrome. Four patients underwent HCT but succumbed to transplant-related infections. There were four cases of post-transplant lymphoproliferative disease treated with Rituximab. Four patients developed non-Hodgkin lymphoma (NHL); two died within a year of diagnosis, one died of other causes 10 years after diagnosis, and the outcome was unknown for one. The outcome for a patient with Hodgkin lymphoma was not available.

HNSCC was notable for 21 occurrences in 11 patients. There were 14 localized cancers requiring resection in 4 patients, including one patient with 11 resections (Figure 3). Seven invasive cancers occurred in seven patients and were treated with different modalities, likely due to each patient's specific needs. Unfortunately, all patients with invasive HNSCC died due to the cancer or therapy-related complications.

Figure 3. Head and neck squamous cell carcinoma (HNSCC) in patients with DC. Cancer treatment records were reviewed for 11 patients with 21 HNSCCs. Affected genes were 1 *DKC1*, 1 *RTEL1*, 3 *TERC*, 2 *TINF2*, 1 *WRAP53*, and 3 unknown. Abbreviations: RT, radiation therapy; chemo, chemotherapy
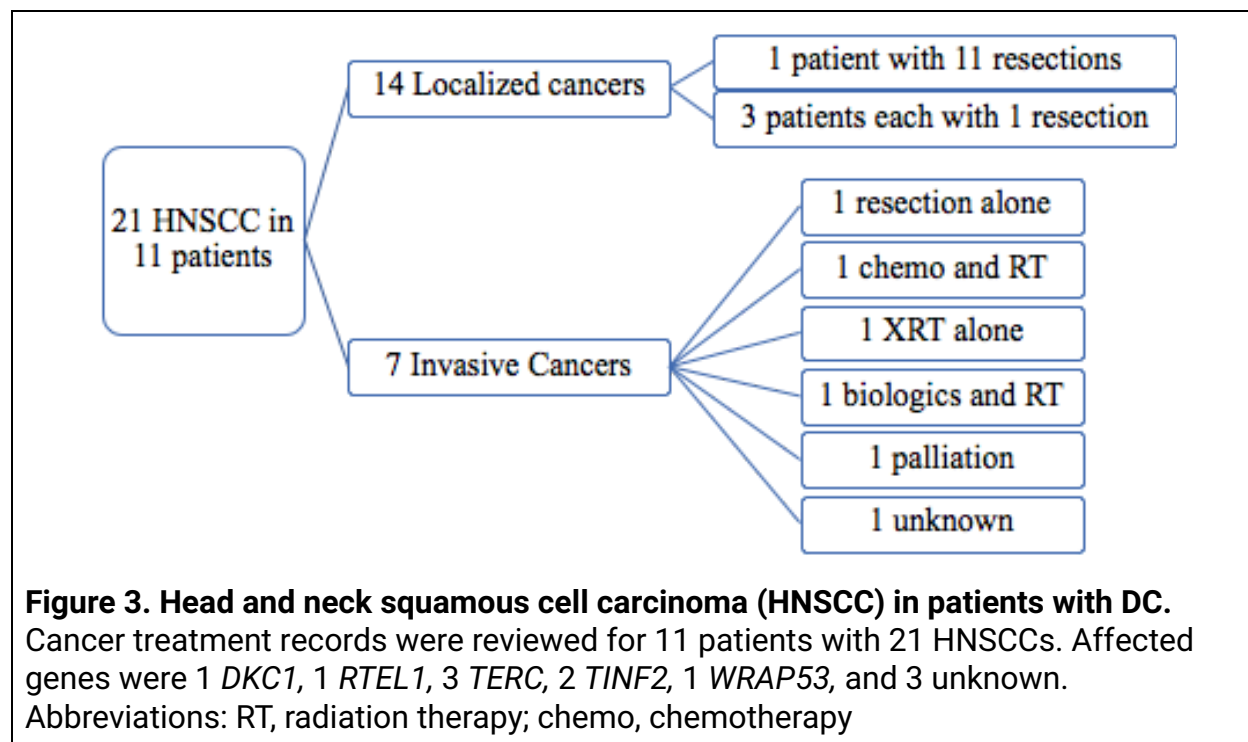
These descriptive data and other case reports suggest that people with DC/TBDs have unique sensitivities to chemotherapy and radiation therapy. Additional studies are needed to improve understanding of cancer therapy-related side effects.

Cancer Prevention and Surveillance

Prospective studies of cancer screening in patients with DC/TBDs have not yet been conducted and are urgently needed. In general, early cancer diagnosis is important to reduce cancer-related morbidity and mortality in patients with DC/TBDs. For example, screening can identify HNSCC when it is still amenable to surgical resection only. Table 2 lists the current recommendations.

Table 2: Cancer screening and prevention methods recommendations for people with DC/TBDs. These recommendations are based on expert opinion as no prospective clinical trials of cancer prevention have been conducted for DC/TBDs. Everyone should also follow the same cancer screening recommendations as the general population. More information on cancer screening in the general population is available at http://www.cancer.gov/cancertopics/screening.

Cancer	Approximate Start Age	Screening Method	Prevention
HNSCC	10 years	Annual oral exam, nasolaryngoscopy	No smoking or drinking alcohol. Good oral hygien See also Chapter 8
Gynecologic	16 years or after first period	Annual gynecologic exam, pap smear, HPV test	HPV vaccine See also Chapter 21
Rectal	18 years	Physical exam, check stool for blood annually	
Esophagus	20 years	Esophagoscopy, as needed	No smoking or drinking alcohol
Liver	Infancy	Liver ultrasound, liver function tests	No drinking alcohol; chelate iron if transfused See also Chapter 19
Skin	Infancy	Dermatology exam, annually	Sun protection See also Chapter 6
Leukemia	Infancy	Complete blood counts, bone marrow aspirate, biopsy	See also Chapter 12
Lung	40 years	Chest X-ray, lung function tests, as clinically indicated	No smoking See also Chapter 14

Summary

Patients with DC meet the criteria for "cancer-prone" with most of the cancers presenting at younger ages than expected and more commonly than expected when compared with the general population. The most common solid tumor is HNSCC, which is amenable to early detection with cancer screening. All patients and/or their families are encouraged to promptly report to health care professionals any changes in their health, and physicians should have a low index of suspicion for cancer in patients with DC/TBDs. Those diagnosed with cancer may need a modified cancer treatment plan that addresses potential therapeutic sensitivities and co-occurring illnesses.

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Chapter 10

Medical Management of Bone Marrow Failure in Telomere Biology Disorders

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Introduction

Bone marrow failure (BMF) is a common complication in telomere biology disorders (TBD), and in particular in classic dyskeratosis congenita (DC). As many as 80% of individuals with DC develop BMF by age 30 years [1, 2]. Many individuals diagnosed with TBD have some degree of abnormality in the complete blood count (CBC) profile which may range from minor findings such as macrocytosis (high mean corpuscular volume [MCV] for age, due to large red blood cells), to mild asymptomatic cytopenias in one or more blood cell lineage, to symptomatic BMF

(also referred to as severe aplastic anemia). Some patients may develop progressive abnormalities (dysplasia) in the bone marrow hematopoietic cells, which may subsequently evolve into myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) [3-5].

The time of onset of BMF is highly variable among individuals. Infants or young children with severe forms of DC may present with progressive BMF early in life even before other signs of DC have appeared, whereas older individuals with TBD (particularly those with variants in *TERC* or *TERT*) may develop blood cell abnormalities much later in life or never. The first cytopenia to appear usually is low platelet count, followed by anemia and/or neutropenia [6, 7].

Definition of Bone Marrow Failure

The diagnosis of BMF is made if the blood counts are persistently below the age-appropriate normal values due to the failure of the bone marrow to produce an adequate amount of normal blood cells. Other causes of low blood counts such as infection, medications, peripheral blood cell destruction or nutritional deficiencies should be ruled out first.

The classification of bone marrow failure in DC and TBD is similar to that for Fanconi anemia and is currently based on the consensus guidelines developed for the management of BMF in Fanconi anemia [8]. Patients who are transfusion dependent for either red blood cells or platelets are considered to have severe disease by default.

Table 1. Classification of bone marrow failure.

Bone Marrow Failure	Mild	Moderate	Severe
Absolute neutrophil count/mm³	1,000 to less than normal for age	500 - 1000	<500
Platelets/mm³	50,000 to less than normal for age	30,000 − 50,000	<30,000
Hemoglobin gm/dL	8.0 to less than normal for age		<8.0

The criteria proposed by Camitta and colleagues for diagnosis of immune aplastic anemia can also be applied to denote severe marrow failure in this setting and is defined as: absolute neutrophil count <500/mm³, platelets <20,000/mm³, and absolute reticulocyte count <60,000/mm³.

In an individual with suspected or diagnosed with TBD, a CBC and bone marrow examination should be performed to determine the baseline hematologic status (whether BMF, myelodysplasia, or an abnormal karyotype are present).

Bone marrow examination should consist of both a biopsy and an aspirate. The biopsy is to assess the marrow architecture and cellularity and an aspirate is to determine whether cells within the bone marrow are morphologically normal or abnormal. A sample of the bone marrow aspirate should be sent for cytogenetic evaluation by G-banding and for FISH studies to look for common clonal cytogenetic abnormalities associated with MDS. Next generation sequencing panels for mutations in myeloid cancer genes can be performed, though their significance in the absence of MDS remains unclear. Some degree of cellular morphologic dysplasia is common in individuals with TBD (and in other IBMFS), therefore the presence of minor dysplastic changes in erythroid, myeloid and/or megakaryocyte lineages should not be misconstrued as diagnostic of MDS. A careful evaluation of the baseline bone marrow and subsequent marrows by a hematopathologist with an expertise in marrow failure syndromes is generally warranted to diagnose MDS in TBD.

Monitoring of Bone Marrow Failure

Periodic monitoring of blood counts and bone marrow are important to assess the progression of disease so that timely and appropriate therapeutic intervention can be initiated.

Individuals with non-DC TBD, particularly in the absence of cytopenias or marrow failure, may not require regular bone marrow examination. Guidelines may change over time as new data on the clinical spectrum, heterogeneity of manifestations, progression, treatments, and associated complications become available. There is no consensus on the frequency of bone marrow examination required in individuals with TBD, and it will depend on the clinical background, physician preference, and patient choice. Here we present a potential algorithm, though guidelines may be modified by a treating hematologist and tailored towards individual patient needs as clinically indicated. Individuals with a strong family history of hematologic malignancy, exposure to chemotherapy or radiation, or with a higher risk germline variant may warrant closer follow up.

For patients with:

1. **Normal blood counts and no cytogenetic abnormality**
   - CBC every 6-12 months
   - Bone marrow aspirate/biopsy and cytogenetic testing should be performed once at baseline. If no abnormalities are present on baseline bone marrow and CBC remains normal it is reasonable to repeat bone marrow only if a cytopenia develops.
2. **Stable but mildly low blood counts and no cytogenetic abnormality**
   - CBC every 3-4 months
   - Bone marrow aspirate/biopsy and cytogenetic testing should be performed at baseline. Depending on clinical background, physician preference, and patient choice, bone marrow can be performed at regular

intervals (1-3 years) or can be deferred unless a drop in blood counts occurs.

3. **Blood counts falling or rising:** More frequent monitoring of CBC ± bone marrow evaluation may be indicated.
   - Blood counts may decrease after an episode of infection due to limited bone marrow reserve. Most often the counts will return to the patient's baseline levels within a few weeks after recovery.
   - In patients with progressively declining blood counts without an apparent cause, further investigations with more frequent CBCs and bone marrows for morphology and cytogenetics is indicated. Appropriate plans for intervention should be in place for progressive BMF, or development of MDS or AML.

4. **Clonal cytogenetic abnormality/s:** Presence of a cytogenetic clone by itself (without morphologic evidence of MDS) does not necessarily indicate a diagnosis of MDS. In our experience with TBD, some patients have clonal cytogenetic changes that have persisted for several years (over 10-15 years) without progression to MDS or leukemia. However, MDS associated chromosomal abnormalities should prompt regular monitoring and high risk changes such as monosomy 7 should prompt referral for hematopoietic cell transplantation (HCT).

   General recommendations for clonal cytogenetic abnormalities:
   - Monitor CBC every 1-2 or 2-3 months based on the stability of the blood counts.
   - Repeat bone marrow (with cytogenetics and FISH studies) every 4-6 months for a minimum of 2 -3 times; if the blood counts are stable and the bone marrow clone is unchanged without progression, and the bone marrow morphology is not diagnostic of MDS, it may be safe to revert to annual bone marrow exams.

   Advice for MDS associated cytogenetic abnormalities:

- If MDS associated cytogenetic abnormalities are detected, appropriate plans for HCT should be in place as patients may evolve rapidly to MDS or leukemia. Chromosome 7 changes (in particular monosomy 7) are associated with high risk of leukemic progression and with poor overall survival in BMF and urgent evaluation should be performed for HCT in this situation.

- Other clonal cytogenetic abnormalities such as loss of chromosome 5, trisomy 8, 11q23 translocation, 20q, and 3q abnormalities are known to occur in patients with MDS and in association with transformation to AML. Presence of such clones would need more frequent monitoring of CBC and bone marrow.

Treatment Options for Bone Marrow Failure

Treatment is recommended in patients with persistent severe BMF such as transfusion dependence or blood counts in the moderate-severe range. Immunosuppressive therapy is not recommended for TBD-associated BMF due to lack of demonstrated efficacy. In contrast to acquired aplastic anemia, patients with TBD have a genetic rather than an immune pathophysiology of marrow failure and do not appear to respond to immunosuppressive therapy [9]. Treatment options for TBD-associated BMF include HCT or androgens.

Hematopoietic Cell Transplant (HCT)

HCT is the only curative treatment for BMF or other hematologic complications (MDS, leukemia) in patients with TBD, and is considered the treatment of choice in eligible patients with severe disease. The ideal donor is a matched related donor, proven to not have TBD by physical and laboratory examinations, mutation testing and/or telomere length assay. HCT from an unrelated or alternative source donor such as haploidentical or umbilical cord can be considered for those lacking a fully matched related donor.

However, poor long-term outcomes have been observed in TBD patients post HCT, particularly related to lung toxicity. HCT, while curative of BMF or MDS/AML, does not address the other organ dysfunction seen in TBD. HCT is discussed further in Chapter 13, Hematopoietic Stem Cell Transplantation.

Androgens

Androgens are anabolic steroids that have been in use for a variety of conditions for over 50 years, including for the treatment of acquired BMF and for Fanconi anemia. The published literature of androgen use in TBD has shown varying results but overall suggests that androgen treatment is a reasonable option in patients to improve blood counts, especially hemoglobin. While patients with severe hematologic disease, as fulfilling criteria for severe AA, would typically undergo HCT, some may not be medically eligible (related to concurrent multi-organ disease), lack suitable donors, or choose not to. Additionally, androgens may be a good option for patients with either moderate or severe unilineage cytopenias that could clinically benefit from increased blood counts. Many patients with TBD show a hematopoietic response to androgens with sustained improvement in hemoglobin, platelets and neutrophil counts. Androgens however, have side effects, and patients with TBD seem to be particularly sensitive to the effects of androgens.

The most common side effects reported with androgens are:

- Virilization (or masculinization in females and children) with facial and pubic hair growth, scalp hair loss, penile/clitoris enlargement, deepening of the voice, and acne
- Behavioral changes (e.g. aggression, mood swings)
- Liver toxicity (increase in transaminase and/or bilirubin)
- Alteration in blood lipid profile resulting in abnormally low HDL and high LDL levels
- Growth spurt in children which may result in premature closure of epiphysis (growth plates) and short adult height

- Liver adenomas, peliosis (blood lakes) in spleen and/or liver, and rarely hepatocellular carcinomas

A baseline CBC, hepatic panel, liver and spleen ultrasound, lipid profile, thyroid function, and an x-ray hand for bone age (in a growing child) should be obtained prior to starting therapy. Once the treatment has begun, two to three months at a constant dose is a fair trial, monitoring for hematologic improvement. After the blood counts have stabilized, the androgen dose may be gradually decreased over the next several months (2-4 or 6 months) to the lowest effective dose required to maintain stable blood counts depending on the patient's side effect profile. Close medical supervision and dose adjustments help to achieve the minimum effective dose with least side effects.

Danazol is a synthetic androgen derivative used in the treatment of TBD-related BMF. One prospective clinical trial using 800 mg (16mg/kg in children <50kg) of danazol showed hematologic responses of 79% and 83% after 3 and 6 months of therapy respectively. The majority of patients had *TERT/TERC* variants, with only four having other TBD-related variants (3 *DKC1* and 1 heterozygous *RTEL1*) and 6 having no variant identified. Frequent adverse effects were elevation in liver enzymes (41%), muscle cramps (33%), edema (26%) and lipid abnormalities (26%). Liver hemangioma developed in one enrolled patient requiring cessation of therapy [10]. Other retrospective studies and case series have also demonstrated the hematologic efficacy of danazol in patients with Fanconi anemia and TBD/DC with no severe or unacceptable side effects [11-13].

Other androgens such as oxymetholone or nandrolone are also used in TBD-related BMF. Masculinization in females often occurs with oxymetholone and may limit its use. A lower dose is generally recommended in TBD than in patients with Fanconi anemia because patients with TBD may be more sensitive to the effects of androgens. One analysis looking at patients enrolled on a long-term cohort study (n=16) who had received androgens (oxymetholone [n=14], nandrolone [n=1], and fluoxymesterone [n=1]) showed an overall hematologic response rate of 69%; the majority of patients had

variants in *DKC1*, *TINF2*, and *RTEL1* (2 autosomal recessive, 2 autosomal dominant) [14].

Danazol may reduce the rate of telomere attrition in patients with TBD. In a prospective clinical trial, 16/21 (76%) patients assessed at 6 months and 11/12 (92%) patients at 24 months on danazol had a gain in their telomere length, over baseline [10]. Of note, this initial analysis was performed using qPCR to measure telomere length, in lymphocytes and granulocytes combined, whereas flow-FISH using lymphocyte subsets is now considered the clinical standard. Since then, two subsequent retrospective studies looking at androgens in TBD patients have been reported using lymphocytes measured by flow-FISH. One study comparing telomere length over time in 10 androgen-treated and 16 androgen-untreated patients showed no difference in telomere attrition between the two groups [15]. The other study of 7 patients with TBD showed telomere length improvement in lymphocytes by flow-FISH in all patients. Discordant findings may relate to differences in the frequency of specific inherited mutations in each study, as those showing elongation in telomere length tended to have more androgen treated patients with *TERT*/*TERC* variants [13].

Points Regarding Androgen Therapy

1. Androgen treatment does not cure bone marrow failure but can produce a sustained rise in the blood counts for the duration of the treatment. In some patients this may be several years (e.g. 10-15 years or even longer).
2. Blood counts do not generally reach normal values with androgen treatment but may improve to the extent that a previously transfusion-dependent patient may no longer need red blood cell or platelet transfusion support.
3. Androgens are likely to be more effective in patients who have some degree of bone marrow reserve than in those whose marrow reserve is severely depleted. Patients on androgen therapy may become refractory over time if their bone marrow hematopoietic cellular content becomes depleted.

4. Androgens do not prevent or delay the progression to MDS or AML, nor is there evidence they drive progression.

In patients who have not shown a response to the treatment after a trial of up to 6 months, androgens should be discontinued. Occasionally, some patients who initially did not respond to one androgen may subsequently respond to a different androgen.

Monitoring for the Side Effects of Androgens

Patients on androgen treatment should have baseline clinical and laboratory evaluations and at regular follow-up visits while on treatment as outlined in the table below.

Table 2. Recommended clinical and laboratory evaluations for patients on androgen treatment.

Parameter	Before treatment	On treatment
CBC	Baseline CBC	Repeat every 4-6 weeks until counts are stable, then 2-3 months
LFT	Baseline AST, ALT, bilirubin, gamma GT	Every 1-2 weeks for first month then every 6 – 12 weeks
Lipid profile[1]	Baseline cholesterol, LDL, HDL, triglycerides	Every 6 – 12 months
Thyroid function[2]	Thyroid binding globulin (TBG)	May repeat TBG annually
Liver/spleen ultrasound	Baseline	Every 6 months
Bone age (children)	Baseline in a growing child	Every 6 – 12 months until growth plates fuse
Endocrine evaluation	Baseline	Annually
Height and weight	Baseline	Every visit

[1] Persistently low HDL and high LDL levels may be of concern for future cardiovascular risk in patients on long-term (2-5 years or more) androgen therapy. These patients should be regularly followed by an endocrinologist. Lipid levels usually return to baseline values within 3-6 months after stopping androgens.

[2] Thyroid function is not affected by androgen treatment but thyroid binding globulin levels are decreased in patients on androgens.

Patients who have not shown response to androgen treatment after adequate trial or who have become refractory to androgen treatment may consider HCT. There is currently no evidence that androgen treatment increases the risk of future stem cell transplant-related complications.

Other Treatments

Prednisone (5 mg/day or every other day) in combination with androgens was used in the past to delay the early closure of epiphyses (growth plates). This use is no longer recommended because there are no data to support its beneficial effects and prednisone can cause avascular necrosis and early bone loss (osteopenia/osteoporosis).

Cytokines: Hematopoietic growth factors such as G-CSF or GM-CSF can achieve temporary improvement in counts and may be useful in patients with persistent neutropenia (neutrophil count <500/mm^3) in the presence of recurrent or serious infection. However, there is theoretical concern that growth factor use may stimulate the proliferation of a pre-existing clone and malignant transformation. Splenic peliosis and splenic rupture has been observed when G-CSF was used in combination with androgens [16]. Therefore G-CSF or GM-CSF is not recommended in combination with androgens in patients with TBD.

Eltrombopag: Very little data exists on the use of eltrombopag in TBD. In one French study looking at eltrombopag in SAA patients, two were later found to have TBD variants, and neither had responded to eltrombopag [17]. In a NIH clinical trial using eltrombopag in MAA, one TBD patient was included who was deemed a responder [18]. Given the lack of evidence to date, eltrombopag should be considered investigational for patients with TBD and administered in the setting of a clinical trial.

Investigational protocols: Investigational protocols may be considered for patients who are not candidates for HCT and who fail to respond to androgen treatment.

Management Guidelines for Bone Marrow Failure

Clinical management of TBD is complex because several systems may be affected simultaneously to varying degrees, and phenotype may greatly differ between patients.

A treatment approach that works for one patient may not be ideal for another. Therefore, the risks and benefits of available treatments should be discussed with a hematologist with expertise in the care of patients with TBD.

A broad general approach to treatment of BMF is outlined below:

At the time of diagnosis of TBD

- The patient should be evaluated and followed by a hematologist for medical monitoring and management. A detailed assessment of all systems should be undertaken (as per the TBD guidelines) to assess the degree of involvement of other systems. A consult with a hematologist with an expertise in TBD should be sought.
- For patients with any degree of cytopenia, treatment options for bone marrow failure should be discussed in case cytopenias progress to the extent of needing treatment. Early discussion with a HCT team with an expertise in transplanting TBD patients may be considered. HLA-typing and genetic mutation testing of family members for TBD should be considered to assess the availability of a potential HCT donor and exclude any potential affected family member as a donor.
- Families should be referred for appropriate medical counseling as some may wish to have more children and may be interested in pursuing prenatal screening. Preimplantation genetic diagnosis (PGD) and selection of unaffected embryo (who is also an HLA match) for the patient can also be considered.

Normal blood counts or mild bone marrow failure or moderate bone marrow failure

- Monitor CBC and bone marrow as described earlier (see monitoring of BMF) until further treatment is needed.

- Continue discussions regarding treatment options. For patients with declining counts, consider referral to HCT team if not already done; however, HCT need not be undertaken until the development of severe marrow failure or MDS/AML.

- A donor should be identified, most preferably an HLA-identical sibling (proven to be telomere mutation negative), but a matched unrelated donor or alternate donor may also be considered as necessary.

- Consider androgen therapy for patients with clinically significant cytopenias.

Severe bone marrow failure

- Consider HCT for eligible patients

- Begin androgen treatment for patients who are not candidates for HCT due to lack of suitable donor, medical ineligibility, high risk transplant stratus, or unwillingness to undergo HCT.

Severe bone marrow failure unresponsive to androgens and high risk for transplant

- Consider cytokines, supportive care, or investigational protocols

MDS or AML

The diagnosis of MDS in a patient with TBD should be confirmed by a hematopathologist with an expertise in these disorders.

No standard effective therapy other than HCT has been established for MDS or AML associated with TBD.

- Patients should be referred for HCT with or without prior induction chemotherapy

- Phase I/II trials may be considered for patients ineligible for HCT

See Chapter 13, Hematopoietic Stem Cell Transplantation for further information on this topic.

Supportive Care

Some patients with TBD may need red blood cell and/or platelet transfusion support prior to initiation of definitive treatment, before therapy becomes effective, or if other treatments have failed. Timely referral to a transplant center for consideration of HCT should be made for patients who become transfusion dependent.

Anemia: Red blood cell transfusion may be unavoidable and have few immediate adverse consequences. However, chronic red blood cell transfusions may adversely affect transplant outcomes.

Patients receiving many red blood cell transfusions should be monitored for iron overload by at least serum ferritin. T2* MRI of heart and liver or other appropriate studies should be performed if organ damage is suspected. Appropriate treatment with iron chelators such as deferoxamine (Desferal) or deferasirox (Exjade) should be initiated if iron overload is present.

Thrombocytopenia: Platelet transfusions may be indicated in patients with severe thrombocytopenia, or in those undergoing invasive procedures or with mucosal bleeding. Amicar or tranexamic acid may be used as an adjunct to platelet transfusions in patients with mucosal bleeding.

Non-steroidal anti-inflammatory drugs, aspirin and other drugs that inhibit platelet function should be avoided in patients with platelet counts $<50 \times 10^9$/L.

Activities carrying high risk of trauma (e.g. contact sports) should be avoided in patients with platelet counts $<50 \times 10^9$/L.

Neutropenia: G-CSF may be considered in patients with severe neutropenia and concurrent infection. G-CSF should not be used in patients on androgens as the risk of splenic peliosis with rupture may be higher with this combination.

Ongoing Clinical Research

Further studies looking at the use of androgens in patients in TBD are ongoing. Research objectives include: use of lower doses of danazol, assessing the use of danazol for other organ dysfunction in telomere disease such as liver and lung fibrosis, and evaluating the effect of danazol and other androgens on telomere length over time.

Studies looking at ways to reduce post-HCT toxicity using reduced intensity conditioning are also currently underway and may result in more favorable long-term outcomes. Additionally, gene therapy for patients with TBD is currently under investigation.

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Chapter 11

Immunologic Complications in Dyskeratosis Congenita and Hoyeraal-Hreidarsson Syndrome

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Introduction

Immune cells are highly proliferative upon stimulation by antigens. Hence, lymphocytes are particularly vulnerable to the effects of telomere dysfunction. An accelerated decline in the number and function of peripheral mature immune cells, a phenomenon known as immune senescence, also contributes to the pathophysiology of DC/HH [1, 2]. Additionally, it is possible that the dysfunction of

the hematopoietic environment, from which immune cells originate, contributes to immune dysfunction in these disorders [3]. Individuals with DC and HH can exhibit a progressive immune deficiency manifesting as increased susceptibility to life-threatening infections, the severity of which worsens with age. Immunologic complications are one of the common features of DC and HH and contribute to the premature mortality seen in these disorders [4]. This chapter summarizes the main immunologic complications observed in individuals wiht DC/HH and the recommendations to evaluate and manage them. Each patient requires their own treatment plan, managed in collaboration with immunologists, hematologists, and other specialists.

Immunological Features of Individuals with DC and HH

Lymphopenia (low numbers of lymphocytes) is the most common immunological abnormality observed in patients with DC and HH (70 % of patients) [1, 2]. A decreased count of B cells and NK cells are the most remarkable signs of these syndromes. In particular, a virtual absence of B lymphocytes is often observed in HH from birth [5-12]. B lymphocytes produce antibodies (immunoglobulins known as IgG, IgM, or IgA) and are part of what is known as the humoral immune system. Low numbers of B lymphocytes is one of the most consistent immunological features of DC/HH and results in hypogammaglobulinemia that can affect all immunoglobulin subtypes (IgG, IgM, or IgA). The antibody response toward specific antigens is sometimes impaired, which may reduce the response to vaccinations. The marked involvement of B lymphocytes in DC and HH is likely due to the additional cell proliferation which B lymphocytes undergo during their development (thus resulting in accelerated telomere shortening) coupled with a shorter life span than T lymphocytes [13]. The T cell compartment is less frequently affected as T cells subsets' absolute number and function is often normal in

DC and HH. However, some patients exhibit a decrease in T cell counts (CD4 and/or CD8 counts), inversion of CD4/CD8 ratio, and a prematurely advanced naive to memory (CD45RA/CD45RO) T cell transition [9]. Abnormality of T cell proliferation to specific antigens (candida and tetanus), and less frequently to mitogens, indicating reduced T cell function, has also been observed [14].

Clinical Presentation of the Immunodeficiency

Individuals with DC or HH often have markedly variable clinical features of immunodeficiency – from severe combined immunodeficiency (SCID)–like presentation in infancy to much milder presentation in adolescents mimicking common variable immunodeficiency (CVID). Opportunistic infections (e.g., Pneumocystis Jirovecii infection or CMV infection) are seen in individuals with T cell dysfunction. The antibody deficiency is associated with recurrent sinus and/or lung infections. The most prominent immune-mediated clinical feature of infant-onset DC or HH is a severe, chronic, non-infectious enteropathy. The histopathological hallmark of the intestinal involvement is the presence of mucosal inflammation and apoptosis (similar to what is observed in gut graft versus host disease) associated with intractable diarrhea. It is unclear if this enteropathy results only from a defect in the renewal of the digestive epithelium or if gut mucosal immune dysfunction also participates in the pathophysiology of this feature. Strikingly, some patients presenting with such enteropathy have benefited from anti-TNF-alpha monoclonal antibody (infliximab or adalimumab).

Immune Evaluation of Individuals with DC and HH

As immune abnormalities can precede the development of clinically significant pancytopenia in individuals with DC/HH, they might be underdiagnosed, undertreated, and lead to premature mortality because of severe infections. Thus, all newly diagnosed patients would benefit from a complete immunological evaluation consisting of

complete blood cell counts as well as a numeration of lymphocyte subsets performed by flow cytometry: CD3+CD4+ and CD3+CD8+ T cells (with naive and memory subsets based on the expression of CCR7 or CD62L and CD45RA), CD56+CD3+ NK cells, CD19+ B cells and CD27+CD19+ memory B cells. Assessment of lymphocyte proliferative responses to the mitogens (mainly phytohemagglutinin) should be performed if the patient's clinical features include a severe combined immunodeficiency (i.e., CD3+ cells below 500 per mm3). Serum immunoglobulin levels (IgG, IgA, and IgM) should be evaluated as well as tetanus, diphtheria, and pneumococcal antibody titers.

Management of the Immune Deficiency

Treatment of patients depends on the clinical presentation and degree of immune dysfunction. Antimicrobial prophylaxis with trimethoprim-sulfamethoxazole (5-6 mg/kg TMP component three times a week) may be recommended for patients at risk for opportunistic infection due to severe T cell lymphopenia. Intravenous (IV) or subcutaneous (SC) immunoglobulin replacement (400 to 600 mg/kg monthly for IV and 100 to 150 mg/kg weekly for SC) is indicated in case of recurrent bacterial infections resulting from low antibody levels. The annual influenza vaccination is recommended, especially in case of pulmonary involvement, even for patients with immunoglobulin substitution, as cellular immunity plays an important role in the resolution of influenza virus infection, and new strains of influenza are typically not represented in the antibody repertoire of immunoglobulin substitution [15].

Immunologic Improvement by Hematopoietic Cell Transplantation

As mentioned above, the immunologic complications observed in DC/HH patients mainly stem from bone marrow failure that compromise the development of a functional immune system. Thus, hematopoietic cell transplantation (HCT, see Chapter

13, Hematopoietic Stem Cell Transplant) is a treatment that can improve the immunological status of the patients. The clinical benefit of a partial reconstitution of the immune system in bone marrow failure syndromes, including DC, has been demonstrated in rare patients who experienced a spontaneous genetic reversion (i.e., the correction of the genetic mutation) in cells from the immune/hematopoietic compartment [16-18]. In these cases, the genetic reversion confers a strong selective advantage of the corrected cells that can, at least in part, reconstitute an efficient immune system and protect patients. This rare phenomenon of genetic reversion appears to be highly beneficial for the patients in terms of immunologic complications. Therefore, by analogy, the severe immunodeficiencies observed in DC-HH patients can be cured by HCT. However, as described in Chapter 13, Hematopoietic Stem Cell Transplant, HCT can be associated with significant complications and long-term concerns and does not correct the non-hematopoietic abnormalities seen in this disease.

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Chapter 12

Myelodysplastic Syndromes and Acute Myeloid Leukemia

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Introduction

Telomere biology disorders (TBDs), specifically disorders associated with short telomeres such as dyskeratosis congenita (DC) are cancer prone conditions. Among these cancers, there is a significantly increased risk of myeloid neoplasms such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). DC/TBD patients who develop MDS/AML need specialized management with unique clinical considerations. In this chapter, we will review MDS/AML in TBDs with a focus on clinical management in adults.

Background

Telomere biology disorders (TBDs), specifically disorders associated with short telomeres such as dyskeratosis congenita (DC) are cancer prone conditions (see also Chapter 9, Solid Tumors). Studies from the National Cancer Institute (NCI) and Johns Hopkins University have indicated an overall incidence of cancer in patients with TBDs to be around 10% [1-3]. Among these cancers, there is a significantly increased risk of myeloid neoplasms such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), as well as squamous cell carcinomas of the head and neck [1].

Telomere length regulation is very tightly regulated in all cells (see also Chapter 2, Why Telomeres Matter). The overall increased frequency of cancer in TBDs is somewhat inconsistent with the theory that telomere shortening-induced replicative senescence and senescence-independent autophagy are mechanisms of tumor suppression [4, 5]. There are several studies in support of the hypothesis that telomere shortening offers protection against neoplastic transformation. Cancers such as malignant melanoma,

chronic lymphocytic leukemia [6-9] have been associated with "long telomere" states, and myeloproliferative neoplasms are associated with *TERT* polymorphisms which elongate leukocyte telomeres [10]. Additionally, targeting the telomerase enzyme in myelofibrosis has shown some efficacy in cancer treatment [11]. However, there are some noteworthy exceptions to this hypothesis. Firstly, loss of tumor suppressor mechanisms such as *p53-p21* or *p16-pRB*-mediated DNA repair can induce genetic instability in cells with critically short telomeres, and thereby induce neoplastic transformation [4]. Additionally, emergence of clonal hematopoiesis in the hematopoietic compartment (one of the sites where telomerase is most active) can confer a fitness advantage to mutant clones on the background of accelerated stem cell pool depletion and can result in evolution of MDS and/or AML through serial acquisition of additional somatic variants [3, 12]. Recent work has shown that telomere erosion leads to formation of unstable dicentric chromosomes and chromosomal crisis, a phenomenon called "chromothripsis" or "Kataegis", which is commonly associated with MDS/AML [13]. Another potential mechanism is adaptive immune dysregulation in TBDs [14], which has been shown to accelerate clonal evolution in other contexts [15]. These studies highlight both stem-cell intrinsic and extrinsic impact of telomere dysfunction in the development of MDS/AML.

Due to the unique nature of TBDs, affected individuals who develop MDS/AML need specialized management with unique clinical considerations. In recognition of this unique entity, the recent iteration of the World Health Organization classifies MDS/AML associated with TBDs as a separate entity under "myeloid neoplasms with germline predisposition and other organ dysfunction" [16]. In this chapter, we will review MDS/AML in TBDs with a focus on clinical management in adults. For practical purposes, MDS and AML are grouped together as 'myeloid neoplasms', and their precursor states such as clonal hematopoiesis and clonal cytopenias of undetermined significance (CCUS) are defined as in Table 1 [12, 16]. Since this is an emerging area, there is limited evidence for TBD-specific clinical decision-making. In describing our

recommendations, we clarify the level and grade of recommendation as outlined in Table 2, adapted from reference [17].

Table 1: Terminology used to describe various types of myeloid conditions.

Clonal hematopoiesis (CH)	Somatic pathogenic variants associated with hematologic malignancy in hematopoietic cells at a variant allele frequency of $\geq$2% in the absence of cytopenias and without any other diagnostic criteria for hematologic malignancy.
Clonal cytopenias of undetermined significance (CCUS)	CH plus cytopenia in one or more lineages.
Myelodysplastic syndromes (MDS)	Group of clonal hematopoietic stem cell disorders characterized by cytopenias, bone marrow biopsy showing dysplasia in $\geq$10% in one or more myeloid lineage, and <20% blasts in bone marrow aspirate.
Acute myeloid leukemia (AML)	$\geq$20% blasts in peripheral blood or bone marrow aspirate.

Table 2: Levels and grades of evidence used in this chapter.

Level of evidence	
Type I	Evidence obtained from meta-analysis of multiple, well-designed, controlled studies. Randomized trials with low false-positive and low false-negative errors (high power).
Type II	Evidence obtained from at least one well-designed experimental study.
Type III	Evidence obtained from well-designed, quasi-experimental studies such as non-randomized, controlled single-group, pre-post, cohort, time, or matched case-control series.
Type IV	Evidence from well-designed, nonexperimental studies such as comparative and correlational descriptive and case studies.
Type V	Evidence from case reports and clinical examples.
Grade for recommendation	
A	There is evidence of type I or consistent findings from multiple studies of types II, III, or IV.
B	There is evidence of types II, III, or IV and findings are generally consistent.
C	There is evidence of types II, III, or IV but findings are inconsistent.

Incidence

In the NCI cohort of individuals with DC/TBD with a 15-year follow-up, the overall incidence of cancer was 10%; 3% for AML and 11% for MDS. The authors also calculated an observed-to-expected (O/E) incidence ratio based on data from Surveillance, Epidemiology, and End Results after adjustment for age, sex, race and birth

cohort. The O/E ratio was 578 for MDS and 73 for AML, while the overall cancer ratio was 4.2 [1]. This suggests that MDS and AML are among the most frequent cancers in DC patients, along with head and neck squamous cell cancers. Further, the median age of presentation for any cancer is approximately 38 (range: 18-63) years; 40 (range: 28-56) years for AML, and 31 (4-73) years for MDS [1]. The median age of presentation in the Johns Hopkins University cohort was higher at 53 (range: 12-71) years [3]. In both datasets, the age-at-onset is younger than expected in the general population.

Genetic Patterns

TBDs are characterized by a unique constellation of multisystemic clinical features such as nail dystrophy, skin pigmentation, oral leukoplakia, bone marrow failure, and/or pulmonary or hepatic fibrosis [18, 19]. Late or adult onset TBDs are often associated with *TERT* and *TERC* heterozygous variants, predominantly presenting with bone marrow failure, idiopathic pulmonary fibrosis, or hepatic disease without the classical DC-associated findings. Individuals with *RTEL1* or *PARN* heterozygous variants seem to present with bone marrow failure less frequently. With respect to MDS/AML, an association with specific germline telomere biology gene pathogenic variants has not been found, which suggests that short telomeres rather than gene-specific impairment may be the main driver of leukemogenesis in these individuals [1]. Larger cohorts are needed to further clarify whether any specific germline telomere gene defect is associated with a higher risk of MDS/AML development, and whether that risk is independent of the two common genetic variants (single nucleotide variants or SNPs, rs2853669 and rs2736100) in *TERT* that may be associated with AML [20, 21].

Clonal evolution and MDS/AML in TBDs are often characterized by acquired monosomy 7, but also trisomy 8, similar to other germline bone marrow failure syndromes [19, 22]. In conventional clinical practice, monosomy 7 is considered a "high risk" abnormality predicting a poor prognosis. Despite these specific abnormal cytogenetic associations, a study showed that the mutational spectrum of TBD-associated MDS/AML is not

significantly different from an unselected population of MDS/AML patients, although the sampled cohort had a relatively small sample size [3]. The somatic mutations include epigenetic regulators (*DNMT3A, TET2, ASXL1*), splicing factor variants (*SF3B1, U2AF1*), DNA repair (*TP53, ATM*), transcription factors (*BCORL1, ETV6, RUNX1*) and cell signaling (*CBL, GNAS* and *MPL*).

It is also worth noting that the rate of clonal hematopoiesis (precursor to MDS/AML) was about three times higher in TBD patients compared with healthy individuals [3]. This suggests that evaluation for CH may identify TBD patients at higher risk of MDS/AML development and should be prospectively studied as a future screening strategy.

Screening

In the absence of prospective data, it is not known whether it is appropriate to screen for MDS/AML, what tools to use for screening, and what is the appropriate age to start the screening in TBD patients. As mentioned above, the reported median ages of AML and MDS presentation in adult patients with TBDs range from 40-52 years. Our approach is to selectively consider screening for MDS/AML in patients <40 years in patients by following blood counts over time. In patients $\geq$ 40 years of age with a documented germline telomere gene variant and short telomeres, we recommend that patients should be screened for clonal evolution through annual bone marrow exam (type IV level of evidence, grade B recommendation). An alternative screening approach is to annually test peripheral blood for myeloid-specific gene variants; however, diagnostic utility is not established, and thus reimbursement from insurance agencies may pose a challenge (type V level of evidence, grade C recommendation). Significant family history of MDS/AML in two or more first or second relatives, and their ages of presentation may provide individualized guidance on screening. Genetic anticipation, that is, occurrence of phenotype at an earlier age when compared to the previous generation due to inheritance of both the telomere gene related mutation and short telomeres, can occur in TBDs and should be considered when making these decisions.

Although there is lack of evidence for either approach (type V level of evidence), we recommend screening because the appearance of clonal evolution may prompt a time-sensitive preparation for allogeneic hematopoietic cell transplantation (HCT) (grade B recommendation) (see also Chapter 13, Hematopoietic Stem Cell Transplantation). Further, the type of allogeneic HCT, including the choice of donor and conditioning regimen, varies and may need to be different for patients with or without clonal evolution [24, 25]. To be specific, TBD patients without clonal evolution may benefit from a conditioning regimen with lower doses of alkylator or total body irradiation and preferably, a total body irradiation-free and alkylator-free regimen which is currently under clinical trial investigation (NCT#01659606). However, appearance of clonal evolution and MDS/AML may necessitate intensification of the conditioning regimen which can lead to increased toxicity in TBD patients [26]. The specific types of cytotoxic therapies used in the treatment of MDS/AML associated with short telomeres are discussed below, both in the HCT and non-HCT setting. The advent of venetoclax plus hypomethylating agent therapy in AML therapy is promising as it offers a relatively less intense alternative without compromising efficacy significantly, but this needs to be studied in patients with TBDs [27].

Management

Initial Therapy

The initial therapy of MDS/AML in patients with TBDs is not well studied and needs to be individualized to the patient. Toxicity from conventional cytotoxic chemotherapy containing anthracycline and cytarabine may be excessive given the short telomere-associated hypersensitivity of rapidly dividing cells to total body irradiation and alkylator chemotherapy [26]. Similar patterns of excess total body irradiation and alkylator chemotherapy-associated toxicity, in particular, delayed count recovery and mucositis, have been described in other germline bone marrow failure syndromes such as Fanconi anemia [28] and Shwachman-Diamond Syndrome [29]. Additionally,

TBD-specific extra-hematopoeitic complications such as pulmonary fibrosis and hepatopulmonary syndrome need consideration prior to aggressive therapies [3]. In addition to standard evaluation, in TBD patients with MDS/AML, we recommend testing for liver stiffness with a magnetic resonance elastrography (MR elastogram) and pulmonary function with spirometry/diffusion capacity testing and high-resolution computed tomography (HRCT) scan prior to initiating MDS/AML-directed therapy (level IV/V, grade B recommendation).

In pediatric and adult MDS patients, HMA remains the standard choice for cytoreduction (BM blast ≤ 10%) followed by an allogeneic HCT in intermediate-high risk patients (type V level of evidence, grade C recommendation) [30, 31]. For AML therapy, intensive cytotoxic therapy is still the standard of care in young, otherwise fit patients. The regimen of venetoclax plus hypomethylating agent therapy (HMA) is promising for older adults with co-morbidities as it offers a relatively less intense alternative without compromising excessively on efficacy [27]. The optimal approach in patients with TBDs presenting with MDS/AML is unknown, but depending on the clinical context and if available, clinical trials with non-cytotoxic targeted therapies and immunotherapy may be offered to patients.

Allogeneic hematopoietic cell transplantation for MDS or AML

TBD patients presenting with MDS/AML should be considered candidates for allogeneic HCT if they fall under the intermediate to high risk categories as assessed by the revised international prognostic scoring system (IPSS-R) for MDS and European LeukemiaNet classification for AML. The presence of short telomeres increase risk for excessive transplant-associated toxicities as has been demonstrated in many studies [24, 32, 33]. Additional organ dysfunction such as pulmonary fibrosis and hepatic disease play a critical role in the choice of conditioning regimen. Agarwal *et al*. have developed a novel conditioning protocol with lower toxicity which is under clinical trial investigation, however this protocol excludes patients with cytogenetic abnormalities associated with MDS and AML (NCT#01659606, see also Chapter 13, Hematopoietic

Stem Cell Transplantation). When clonal evolution occurs, we recommend choosing a relatively more intensive but still reduced intensity conditioning (type V level of evidence, grade B recommendation). The choice of donors is also especially relevant as related or sibling donors can be carriers of germline variants without obvious phenotypic manifestations. Any potential related donor should undergo telomere length testing or genetic testing if a mutation is known. Choice of stem cell source is highly contextual and depends upon disease status prior to transplant. In pediatric patients, long-term effects of graft versus host disease are especially relevant, and bone marrow is the preferred stem cell source. Optimal choice of conditioning, donors, and stem cell source needs to be systematically studied. Post-HCT care should involve monitoring for development of infections and secondary neoplasms such as squamous cell cancers, including appropriate cancer surveillance and vaccinations. Immune reconstitution may be impaired in some of patients with TBDs due to the inherent T cell immunodeficiency, placing them at risk for infections. Also, the rates of transplant-associated secondary malignancies, in particular head and neck, and genital squamous cell cancers are higher and need periodic screening (see also Chapter 9, Solid Tumors) including preventive measures such as human papillomavirus vaccination.

Summary

In summary, surveillance and intervention for MDS/AML is a challenge for patients, families, and medical professionals. The management is complicated by other TBD-associated medical problems and potential treatment-related toxicities. Future research efforts should include studying the biology of clonal evolution in the hematopoietic system of patients with TBDs. Single-cell technologies may clarify the question whether clonal evolution occurs specifically in hematopoietic stem cells with critically short telomeres and DNA repair defects, or emerge as separate clones due to proliferative advantage conferred as a consequence of stem cell pool depletion. Prospective studies evaluating optimal screening methods for MDS and AML in TBD patients and their prognostic value are also necessary. Although the focus should be on

prevention due to the challenges of management and poor outcomes associated with MDS/AML in TBDs, efforts should also be directed towards novel non-cytotoxic drug discovery.

Acknowledgments

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Chapter 13

Hematopoietic Stem Cell Transplantation

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Note: Because of a lack of data to support differential management, in this chapter we do not distinguish classic dyskeratosis congenita versus other telomere biology disorders (for example Hoyeraal-Hreidarsson syndrome, Revesz syndrome, or aplastic anemia with very short peripheral blood cell telomere length, and/or with mutations in telomere biology genes). The comments are intended to apply generally to individuals with telomere biology disorders.

Introduction

Hematopoietic cell transplantation (HCT) can cure blood defects — bone marrow failure (BMF), myelodysplastic syndrome (MDS), and leukemia — in patients with dyskeratosis congenita (DC). However, HCT does not cure the other problems of DC. Early experience in HCT for DC was characterized by high morbidity and mortality, and raised concerns that conventional transplant regimens accelerated other disease manifestations in DC patients. HCT outcomes have improved in the past decade with advances in diagnosis, donor matching and supportive care, and prospective multi-center trials of reduced-intensity, disease-specific regimens.

History

Case reports in the 1980s and 1990s demonstrated that aplastic anemia (i.e., bone marrow failure) in DC could be cured with HCT [1-11] (reviewed in de la Fuente and Dokal [12]). However, the overall results in this era were dismal, with a 5-year overall survival of approximately 45%, and no long-term survivors of unrelated donor HCT [12, 13]. More than half of all patients died within 4 months of the HCT procedure, most often due to infections, graft failure, or graft versus host disease (GVHD) [12-15].

A striking increase in fatal lung and vascular complications was noted, attributed to both predisposition to pulmonary and endothelial disease in DC patients, and heightened sensitivity to cytotoxic chemotherapy and radiation used in the conditioning regimens [2, 7, 8, 12]. Other factors contributed to poor outcomes. The interval from onset of BMF to transplant was often years [6], and identification of DC sometimes went undiagnosed until after HCT, because the clinical syndrome was not recognized, and genetic or functional testing was unavailable [5].

With increased awareness, new diagnostic tests, and the application of lessons learned from reduced intensity conditioning (RIC) in the DNA repair disorder Fanconi anemia (FA) [16], HCT outcomes have improved for patients with DC in the past 15 years. In a retrospective study of data reported to the Center for International Blood and Marrow Transplantation Research (CIBMTR), the 5-year probability of overall survival for DC patients undergoing HCT from 2000-2009 was 65% [13]. Similarly, in retrospective reviews of DC transplants using RIC regimens after 2000, approximately two-thirds of patients were alive at 5 years, including survivors of unrelated donor and cord blood transplants [14, 15, 17]. The improvement has been attributed to reduction or elimination of alkylating agents (such as cyclophosphamide, busulfan, melphalan, thiotepa) and radiation in preparative regimens, and an increasing use of fludarabine- and antibody-based immunosuppressive conditioning [17-25].

Better outcomes are also the result of improved supportive care of transplant patients, expanded availability of alternative donors and umbilical cord blood grafts, and advances in molecular human leukocyte antigen (HLA) matching techniques. Disease-specific, prospective HCT trials are underway for DC patients [17, 26]. These aim to exploit the telomere maintenance and cellular replication defects in DC patients, and ask whether minimally toxic conditioning regimens will permit successful engraftment. One can anticipate that outcomes will continue to improve in HCT for DC, with new knowledge and coordinated efforts aimed at decreasing adverse effects, and increasing overall length and quality of life for patients.

Proceeding to HCT

Diagnosis

Patients with DC can present with highly variable signs and symptoms, from classic findings in children to isolated hematological abnormalities in adults. Establishing the diagnosis of DC as the cause of the patient's hematological problems has major

implications for how the transplant should be conducted. Therefore, a thorough investigation for DC should be conducted in all patients with BMF and MDS (and some patients with leukemia) who are being evaluated for HCT (see Chapter 3). With the availability of telomere length testing and BMF gene panels, it appears that there may be a growing number of patients diagnosed with telomere biology disorder (TBD) with or without an identified germline pathogenic variant.

It is also important to note that DC is not only a blood disorder, but rather, a systemic disorder that affects the entire vasculature and different organs including eyes, lungs, liver, and gut. The degree of systemic disease is variable among patients; it can be minimal in some patients even in adulthood and can be advanced in early life in other patients. This systemic aspect of the disease affects the transplant decision in two ways: (1) allogeneic HCT is curative for the blood disorder in DC/TBDs, but does not prevent or treat the progression of other systemic disease, and (2) if advanced, the systemic disease may significantly complicate or ultimately contraindicate HCT.

Timing of HCT

In general, the timing of HCT depends on several factors, including:

1. The nature of the patient's hematological problem and its severity
2. The degree of HLA match and the type of donor graft available to the patient
3. The patient's age
4. The patient's overall clinical condition including pulmonary and hepatic status
5. The transplant physician's recommendation
6. The parents' or patient's decision

Indeed, these six elements all come together with regards to the decision to proceed to allogeneic HCT. Here are two examples:

1. A 5-year-old patient with severe BMF, no other physical signs of clinical problems, and an HLA matched unrelated donor should proceed to HCT

2. A 22-year-old patient with moderate BMF, with pulmonary fibrosis, and no matched donors may not be recommended to proceed to HCT

The Age Factor

Over the years, several studies of allogeneic transplantation in non-malignant hematologic disorders have consistently shown that "younger is better" for HCT. Specifically, children younger than 10 years of age have a superior outcome than patients older than 10 [27-29]. It is also relatively accepted that the risks of progression of systemic disease increase with time. These two facts put together bring into the decision-making process a concept that has been used in the blood disorder thalassemia for a long time, that of "pre-emptive transplant." An illustrative and unanswered question is: should a child with DC who has a good donor go to transplant before the age of 10 years, regardless of bone marrow status and blood counts?

The Hematologic Status

More than 80% of patients with classic DC will manifest BMF (defined as one or more peripheral cytopenias) by age 30 [30]. DC patients have a high risk of MDS (>500-fold over the general population) and acute leukemia (73-fold over the general population) [31].

Results of HCT are generally better for patients who are younger and for patients with BMF as compared to older patients or patients with MDS/AML. HCT is curative for BMF in DC, and in theory eliminates the risk of MDS or leukemia originating from the patient's blood cells. Lastly, the risk of graft failure is also higher in patients who have received a higher number of red blood cell or platelet transfusions.

These factors argue in favor of early intervention with HCT for DC patients manifesting hematologic defects, prior to significant transfusion exposure or evolution to MDS or leukemia. However, HCT is associated with a risk of transplant-related death of at least 15% and a risk of chronic GVHD of at least 10%. These risks are likely to be higher in DC

patients compared to other patients because of disease-associated co-morbidities such as lung and liver dysfunction, which adversely influence the HCT outcomes.

Indications for HCT

The following are considered absolute and relative indications for HCT in DC/TBD patients:

Absolute Indications

- **Severe cytopenias:** Defined as hemoglobin < 8 g/dL; absolute neutrophil count (ANC) < 500/mm^3; platelets < 20,000/mm^3; or requiring red blood cell or platelet transfusions to prevent significant symptoms of low hemoglobin or platelets. Immunosuppressive therapy used for idiopathic aplastic anemia will not cure BMF in patients with DC and should not be tried in this situation. Alternative treatments such as androgens or hematopoietic growth factors may be tried as temporizing measures, but for those without contraindications to HCT and with access to a suitable donor, it may be advisable to proceed to HCT without such a trial.
- **High-risk MDS and acute leukemia (that is, high-risk chromosomal abnormalities or marrow blast count >5%):** May require chemotherapy before HCT, depending on the practice of the transplant center.

Relative Indications

- **Moderate cytopenias:** If there is evidence of progression toward transfusion dependence, one may pursue HCT when a donor/graft with a suitable degree of HLA compatibility is available. Alternatively, it is reasonable to consider a trial of androgen therapy prior to proceeding with HCT.
- **Low-risk MDS (morphologic bone marrow dysplasia with no chromosomal abnormalities or with low-risk chromosomal abnormalities):** Depending on

donor availability, it may be favorable to proceed to HCT, given concerns for clonal evolution, rather than continue observation or trial androgen therapy.

Exclusions

In general, to undergo HCT, the patient must not have:

- Uncontrolled bacterial, fungal, or viral infection
- Severe organ dysfunction, such as lungs and liver
- An active pregnancy

Individual circumstances and specific conditioning regimens may permit consideration of HCT in patients with some of these conditions, and should be discussed with the transplant physician.

In summary, the decision on the timing of HCT timing for each DC patient is dependent on patient age, clinical condition, hematologic status, and donor availability, as well as the physician's and patient's assessment of relative risks and benefits.

Assessment and Planning for HCT

Referral to a Transplant Center

Because of disease-specific peri-transplant and long-term care considerations, and need for a tailored RIC regimen, patients should obtain a formal evaluation at a transplant center experienced in conducting HCT for DC. To determine the experience of a transplant center, the physician or patient may wish to ask the questions listed in Box 1. If a preferred transplant center is "out of network" for the patient's insurance, it may be possible to advocate for coverage of care through coordinated efforts of the patient, physician, and the expert transplant center. A similar approach is advised for international patients working with government or private health care insurance.

1. How many allogeneic DC transplants has your center performed? How many in children? How many in adults? How many have survived beyond one year?
2. How many unrelated donor transplants on DC patients has your center performed in the prior calendar year?
3. What specific regimen(s) does your center offer/recommend? (Obtain the doses of each therapy, graft types, and GVHD prophylaxis.) Is this regimen part of a trial?
4. What is your center's long-term follow-up plan for DC patients who undergo HCT?

Box 1. Transplant center interview questions. Adapted from "Fanconi Anemia: Guidelines for Diagnosis and Management"; 3rd edition, 2008; Chapter 10, Table 3; with permission from the Fanconi Anemia Research Fund.

There is no "standard" HCT regimen for DC that is used across centers; each transplant center may offer a different regimen for HCT, based on their own experience. Although this is not unusual in the practice of HCT, it can be unsettling for patients and families, who may be in the position of having to decide between complex medical regimens, usually without a medical background to guide them. In recent years there has been an effort to develop multi-institutional clinical studies employing consistent regimens for each HCT indication in DC (e.g., clinicaltrials.gov/ct2/show/NCT01659606) [26]. In the future, it is hoped that these types of coordinated efforts will yield more rapid advances in knowledge, which in turn will lead to more uniform standards of care among transplant centers.

Patient Assessment

Time and advanced planning are required to gather the information needed for a comprehensive pre-transplant evaluation. For DC patients, such an evaluation will involve the following elements:

Past Medical History

Because of the variability of DC clinical features, a thorough history is required to elicit factors that may complicate HCT. In particular, history should be obtained regarding infections, blood transfusion requirements, and use of prior therapies such as androgens and hematopoietic growth factors. Prenatal, birth, and developmental history, as well as neurologic, ophthalmologic, dental, gastrointestinal, pulmonary, hepatic, gynecologic/urologic, and oncologic conditions should be reviewed in detail. Prior surgeries and medical treatments, allergies, and current medications, including vitamins, supplements, and herbal therapies, should be detailed.

Family History

The family medical history is extremely important. Without exception, any family members being considered as potential HCT donors must undergo telomere length analysis and genetic testing (if the affected gene is known in the patient), to determine disease risk and suitability as a donor. It has been shown that family members who appear to be completely healthy and without any manifestations suggestive of DC may still carry a pathogenic germline genetic variant associated with DC, and may not be suitable HCT donors [32]. Moreover, in families with TBDs, short telomeres can be inherited independent of the genetic variant [33]; this raises the unanswered question of whether a well-matched unrelated donor is preferable to a fully matched related donor who does not carry the DC-associated gene variant but has short peripheral blood cell telomeres.

Social History

Behavioral, school, and work performance issues should be reviewed. Alcohol and tobacco use should be examined because of elevated risk of cancer, liver, and lung disease, both early on in the post-transplant period and long-term.

Physical Examination

Prior to HCT, the physician should systematically assess for physical abnormalities associated with DC that may alter the risk or plan of transplant therapy. The general examination should include particular attention to establishing a baseline for each organ system. This may include:

- Neurological imaging to screen for brain cysts, white matter changes, and calcifications
- Ophthalmological evaluation for retinal bleeding or exudate, and lacrimal duct obstruction
- Oropharyngeal inspection for precancerous lesions, general dental health, and infection risk
- Pulmonary function testing, with measurement of oxygen saturation diffusion capacity of the lung for carbon monoxide (DLCO), and imaging for pulmonary fibrosis or arteriovenous malformations
- Gastrointestinal status including liver function, and evaluation for evidence of cirrhosis, alimentary canal strictures, enteropathy, or gut bleeding
- Urogenital examination for urethral strictures or precancerous lesions
- Cutaneous inspection for baseline skin pigmentation and nail abnormalities, or precancerous skin lesions

The Donor Search

The compatibility of a patient and donor for HCT is determined primarily by their degree of donor/recipient HLA matching.

HLA antigens are encoded by several genetic loci (chromosomal regions), of which each individual has two copies or "alleles". The loci of primary importance are HLA-A, HLA-B, and HLA-DRB1. A "6 out of 6 match" refers to a match at both alleles for all three of these loci. Two additional loci of importance for HCT are HLA-C and HLA-DQB1, and identity at all five of these genetic loci yields a "10 out of 10 match." Donor/recipient

mismatches may or may not be acceptable for HCT, depending on several factors, including which specific HLA locus is mismatched and the type of donor or graft.

Determining whether the patient has a suitable donor is important for medical management decisions, even in the absence of an obvious short-term need for HCT. Therefore, it is essential that patients, siblings, and parents undergo HLA-typing as soon as the diagnosis of DC is made. A patient has a 25% chance of being HLA-identical to a full biological sibling. It is far less likely, but possible, for a parent to be a complete HLA match.

There is no given lower age limit for a potential sibling donor; infants can be used as sibling donors. However, because the number of cells transplanted per unit recipient weight correlates with success of engraftment, it may be difficult to use a sibling donor who is much smaller than the patient.

Generally speaking, a matched sibling is an ideal donor in that (1) there is a higher degree of shared genetic identity with the patient, which reduces the risk of GVHD; and (2) usually a sibling is readily available for donation, reducing the complexity and delays in transplant scheduling. The potential drawbacks of using sibling donors for HCT in patients with DC are (1) the sibling may be a silent carrier of the genetic variant causing the disease; and (2) the sibling may have inherited short telomeres, and hematopoietic stem cells may not be ideal for transplantation.

Because of these issues, all potential sibling donors should undergo a complete blood count, telomere length testing and genetic testing, whenever possible. When there is uncertainty, a bone marrow examination should be performed on the donor to assess for hypocellularity or dysplasia.

If a sibling donor is unavailable, searching for an unrelated donor involves comparing the patient's HLA typing to information stored in worldwide donor registries. A preliminary donor search can be performed by a transplant center within a few days and without cost to the patient. The availability of stored umbilical cord blood (UCB) units

that may be used for HCT is also determined this way. Again, it is essential that the availability of potential donors be determined as soon as the diagnosis of DC is made. In addition to family HLA typing, a preliminary search of existing registries for potential unrelated donors should be performed very early after diagnosis.

A formal unrelated donor search involves determining the willingness, compatibility, and suitability of one or more adult individuals to donate blood or bone marrow to a specific patient. Because it involves blood tests including high resolution HLA typing of potential donors, there are costs to the patient or insurance. The process of identifying a suitable donor can take anywhere from several weeks to months. Once a donor has been identified and the decision is made to proceed with HCT, it may still take several weeks to schedule the donor collection and complete the necessary pre-transplant evaluation and testing. Therefore, early planning is required to prevent delays in HCT.

In some cases, a haploidentical matched donor, a donor who matches at half of the tested HLA loci, may be considered. This is typically a parent or close relative. This type of stem cell donor has the advantage of being readily available and may be considered for patients with very challenging unrelated donor searches who have few unrelated donors with acceptable HLA matches. As a related donor they must also be screened similarly to a sibling donor as above. The potential drawbacks of using haploidentical donors is increased risk of GVHD and potentially slower return of a normal immune system after HCT. To prevent graft versus host disease, these stem cells require additional manipulation and treatments discussed later in this section like *ex vivo* T depletion or post-transplant cyclophosphamide. At the time of this writing experience with the use of haploidentical donors in DC is very limited [34, 35].

The Graft

The graft is the blood or bone marrow product containing the hematopoietic stem cells obtained from the donor for infusion into the patient. Various types of graft can be used:

1. **Bone marrow (BM).** Liquid bone marrow, similar in appearance and consistency to blood, is typically removed from the pelvic bones of donors via needle aspiration. The donor is typically put under general anesthesia for this procedure. The amount removed is dependent on the size of the patient, but ranges from 300–1200 milliliters (10–40 fluid ounces). The BM is filtered and may be further manipulated based on the donor and recipient ABO blood types and recipient size.

2. **Peripheral blood stem cells (PBSC).** Granulocyte colony stimulating factor (GCSF) is given to the donor to mobilize hematopoietic stem cells from the marrow into the peripheral blood. The donor undergoes pheresis, which entails: (1) collection of blood via intravenous catheters, (2) separation and harvesting of white blood cells (which contain the mobilized stem cells), and (3) return of the remaining blood components to the donor. The donor is awake for the procedure, which may require multiple sessions over a few days. PBSC have the potential advantage of improved engraftment compared to bone marrow, but may be associated with higher risk of GVHD.

3. **Umbilical cord blood (UCB) cells.** UCB is rich in hematopoietic stem cells. It is collected from the umbilical cord and placenta immediately after birth, HLA typed, and frozen at specialized blood banks. These banks serve as repositories for UCB units to be dispensed as needed for patients requiring this graft source. The potential advantages of using cord blood for transplantation are that it is readily available, and there is a decreased risk of GVHD. Therefore, less than perfect HLA matching at HLA-A, -B, and -DRB1 is acceptable. In the United States, it is estimated that UCB units mismatched at one or two HLA loci are available for almost all patients younger than 20 years of age and for more than 80% of patients 20 years of age or older [36]. The disadvantage of UCB is that the volume of the product (and therefore the stem cell "dose") is fixed and may be insufficient. In this case, infusion of more than one UCB unit (a double UCB transplant) may be required. When obtained from a public bank, one cannot obtain more stem cells from the same donor. There may also be a higher risk of

graft failure and certain post-transplant viral infections with UCB transplants because of fewer mature immune cells (T lymphocytes) in cord blood.

The choice of a BM, PBSC, or UCB graft for a given patient will depend on several factors including:

1. Urgency of HCT
2. Degree of HLA match for a family donor versus unrelated donor versus UCB donor unit(s)
3. Regimen-specific or transplant center requirement or preference
4. Donor preference (BM versus PBSC donation)
5. Clinical considerations, most notably patient age and history of infections
6. Donor/graft-specific considerations (for example, the age, parity, and cytomegalovirus (CMV) status of the donor, or the cell count of the available UCB unit[s])

Conditioning Regimen

The conditioning regimen (also known as preparative or cytoreductive regimen) is the process by which the patient is treated with chemotherapy, radiation, and/or immunosuppressive drugs to allow engraftment of the donor hematopoietic stem cells. The "intensity" of a conditioning regimen refers to how aggressively the combination of agents depletes the blood-forming and immune cells of the patient. A higher intensity conditioning regimen more reliably enables engraftment of donor cells, but also causes increased toxicity and side effects. An ideal conditioning regimen would subject the patient to the least toxic agents (or no agents at all), and would achieve full replacement of the patient's blood and immune cells, as well as eradication of any dysplastic clones or leukemia cells.

Based on historical evidence showing an unacceptable rate of toxicity and death, fully myeloablative regimens consisting of high dosages of radiation or alkylating agents should not be used to treat patients with DC. Although higher intensity conditioning

regimens may be warranted to eradicate MDS or leukemia, the focus of current trials is to decrease short- and long-term complications by minimizing conditioning intensity as much as possible for DC patients with BMF. An open question is whether patients with MDS/leukemia should be treated with chemotherapy before HCT, or whether they should proceed directly to HCT, as has been described in FA [37].

Relatively few agents are used in reduced intensity regimens for DC, but the combinations and dosages can vary significantly between transplant centers. The major classes of agents, as well as their typical dosages and range of toxic effects are listed in Table 1. At the time of this writing, there is no standard or "consensus" conditioning regimen for patients with DC, and therefore the physician and patient should give detailed consideration to the different regimens being offered at the centers where the patient is being evaluated for HCT. It is also important to note that given the high variability in symptoms and complications affecting different individuals with DC, it is unlikely that there will be one ideal regimen for all patients.

Table 1. Conditioning agents.

Radiation	Physically damages DNA and thereby kills and/or prevents division and growth of patient cells
	Very effective in destroying host blood and immune cells in preparation for donor stem cell engraftment
	Toxic effects are not specific to blood and immune cells: there are dosage-related toxic effects on all organs/tissues that are exposed
	Usually delivered to the whole body (TBI=total body irradiation); sometimes dose is focused on lymphoid organs (TLI=total lymphoid irradiation)
	Myeloablative dose is 1350-1400 cGy (centigray) total in several fractions
	Reduced intensity doses are approximately 200-400 cGy.
Alkylating Agents (examples include cyclophosphamide, busulfan, melphalan, and thiotepa)	Chemically modify and damage DNA, thereby killing and/or preventing division and growth of cells
	Very effective in destroying host blood and immune cells in preparation for donor stem cell engraftment
	Toxic effects are not specific to blood and immune cells: there are dosage-related toxic effects on multiple organs
	High-dosage ranges: cyclophosphamide 120-200 mg/kg total; busulfan 12.8-16 mg/kg total; melphalan 140-180 mg/m2 total
	Reduced intensity dosages: cyclophosphamide 20-50 mg/kg total; busulfan 0.8-3.2 mg/kg total; melphalan 70 mg/m2 total
Fludarabine Phosphate	Interferes with DNA synthesis and thereby kills and/or prevents division and growth of patient cells
	Very effective in destroying host blood and immune cells in preparation for donor stem cell engraftment
	Toxic effects are largely limited to blood and immune cells, because the intravenously administered drug has limited penetration into other tissues

	Major component of reduced intensity conditioning regimens
	Dosage is typically 120 – 200 mg/m^2 total
Antibodies	Bind to and promote the destruction and clearance of hematopoietic and immune cells
	Long-lasting and powerful immunosuppressive agents; can destroy not only the donor immune cells, but depending on dosage and schedule, can deplete the immune cells in the graft can create serum sickness-like reactions in the short-term; other toxic effects are limited to hematopoietic and immune cells
	a. Anti-thymocyte globulin: produced from different sources (horse or rabbit immune globulin raised against human immune cells; or rabbit immune globulin raised against human lymphocyte cell lines)
	i. Long track record of use in HCT ii. Limited by heterogeneity of formulations and lack of availability of particular formulations in different parts of the world
	b. Anti-CD52 antibody (alemtuzumab): humanized monoclonal antibody causes rapid, profound and sustained lymphocyte depletion
	c. Rapid, profound and sustained lymphocyte depletion
	d. May be associated with increased risk of viral reactivations/infections post-transplant
	e. May be associated with decreased risk of GVHD

Graft Versus Host Disease Prophylaxis and Treatment

All patients undergoing allogeneic HCT are at risk of GVHD, which occurs when the immune cells in the donor graft recognize the patient's tissues as "foreign", and cause

inflammation and cell destruction. The two phases of GVHD — acute and chronic — are characterized by different symptoms (Table 2).

Table 2. Manifestations of GVHD. From "Fanconi Anemia: Guidelines for Diagnosis and Management"; 3rd edition, 2008; Chapter 10, Table 8; with permission from the Fanconi Anemia Research Fund.

Acute GVHD	a. Skin (maculopapular rash to generalized erythroderma to desquamation and bullae)
	b. Liver (hyperbilirubinemia)
	c. Gastrointestinal system (secretory diarrhea, abdominal pain, ileus, hemorrhage, nausea/vomiting)
	d. Ocular (photophobia, hemorrhagic conjunctivitis, pseudomembrane formation, and lagophthalmos)
Chronic GVHD	a. Skin (lichen planus, scleroderma, maculopapular rash, hyperkeratosis, hair and nail loss)
	b. Liver (cholestasis, absent bile duct syndrome, cirrhosis, portal hypertension, hepatic failure)
	c. Gastrointestinal system (dysphagia, failure to thrive, aperistalsis, malabsorption syndrome)
	d. Lung: obliterative bronchiolitis (restrictive/obstructive airway disease)
	e. Sicca syndrome (keratoconjunctivitis sicca with burning, photophobia, irritation, pain; oral dryness, pain, lichenoid lesions, gingival atrophy, dental caries)
	f. Vaginitis, vaginal dryness/strictures
	g. Pancytopenia; eosinophilia
	h. Serositis (pleural, pericardial, joint effusions)
	i. Myofasciitis

GVHD is a major cause of morbidity and death after HCT, and the risks of GVHD are higher in unrelated or haploidentical donor PBSC or BM transplants compared to sibling

donor or UCB transplants. Chronic GVHD is of particular concern as it targets tissues that are often already affected in DC patients, and so may accelerate liver or lung failure, malignancy, or other disorders. Several strategies are used to decrease the risk of GVHD, some of which may be preferable in DC patients:

1. **Calcineurin inhibitors:** Cyclosporine A (CSA) and tacrolimus (FK506) are immunosuppressive agents that diminish the response of immune cells to foreign antigens, and are mainstays of GVHD prophylaxis. CSA or FK506 is used for several months after HCT, typically in combination with one or more other GVHD prophylactic strategies described below. The side effects and toxicity profiles of calcineurin inhibitors make them suitable for use in HCT regimens for DC patients.

2. **Methotrexate (MTX):** MTX is given for several doses in the days immediately following graft infusion. Because it inhibits DNA synthesis, it destroys donor immune cells that otherwise divide rapidly in response to the patient's "foreign" antigens. MTX effects are not specific to immune cells. It may cause mucositis, pulmonary fibrosis, and other cytotoxicity, so it is preferable to avoid using it as GVHD prophylaxis in DC patients.

3. **Mycophenolate mofetil (MMF):** MMF also inhibits immune cells in the donor graft but without significant toxicity to other cell types. It is given for several weeks after HCT. The side effects and toxicity profiles make MMF suitable for use in DC patients.

4. **Graft modification**
   a. ***Ex vivo* T cell depletion:** Reduction of T cells in the donor graft significantly reduces the risk of GVHD without exposing the patient to pharmacological toxicity. This may be accomplished by specifically removing T cells from the graft (e.g., "alpha/beta T cell depletion"), or enriching stem cells from graft ("CD34+ selection") in the laboratory prior to infusion into the patient. T cell reduction may permit a shorter duration or elimination of GVHD prophylaxis. T cell depleted grafts have been used

successfully in DC patients [34, 38]. The main risks of T cell depletion are graft failure and an increased susceptibility to viral infections. T cell depletion is not available at all transplant centers.

b. *In vivo* **T cell depletion:** Anti-thymocyte globulin (ATG), alemtuzumab, or other anti-lymphocyte antibodies given as part of the conditioning regimen may persist in the patient after infusion of the graft and so effectively result in T cell depletion. This may be used as a strategy in the setting of haploidentical stem cell transplant in some patients to avoid the use of cyclophosphamide. The degree of GVHD protection afforded by this strategy is difficult to measure and is likely to be highly variable between patients. Like ex vivo T cell depletion, major risks may include increased graft failure and viral infections.

c. **Post-transplant cyclophosphamide:** In the setting of haploidentical stem cell transplant cyclophosphamide (Cytoxan) can be given for several doses after the stem cell infusion to destroy donor immune cells that otherwise divide rapidly in response to the patient's "foreign" antigens. Cyclophosphamide is an alkylating chemotherapy that causes DNA damage that is not specific to immune cells. It may cause mucositis, pulmonary fibrosis, and other cytotoxicity, so doses must be reduced in patients with DC. Experience with using this approach is limited at the time of this writing and largely based on experience in other chemo-sensitive diseases [39, 40].

Despite preventive measures, patients may still develop GVHD, ranging in severity from limited skin involvement to life-threatening multi-organ failure. Corticosteroids such as methylprednisolone are first-line therapy for GVHD, and adequate control may require long-term immunosuppression. In DC patients with GVHD, consideration should be given early on to strategies that minimize cumulative exposure to high-dose, systemic corticosteroids, to reduce additive effects on musculoskeletal, endocrine, and other organ systems (see also Chapter 22).

Transplant Care Timeline

The timeline of HCT for DC patients can be broken down to 4 periods:

1. Conditioning/preparative therapy
2. Graft infusion and supportive care until engraftment
3. Post-HCT care
4. Long-term care (Table 3)

Patients are usually hospitalized from the period of conditioning through engraftment, approximately 4-6 weeks, followed by outpatient post-HCT care over the subsequent 9-12 months.

Conditioning/Preparative Therapy

Prior to or upon admission, a central venous catheter is placed to enable routine blood sampling and supportive care during the HCT procedure. In the 7-10 days prior to graft infusion, the patient is hospitalized and the conditioning regimen is administered. During this period, depending on the regimen, patients may experience immediate side effects such as nausea, vomiting, fever and fatigue. Medications to control these symptoms and prevent infections are administered. GVHD prophylaxis may begin during this time.

Graft Infusion and Supportive Care Until Engraftment

The day of the graft infusion is termed "day 0." Hydration and medications to prevent infusion reactions are administered. The graft is administered intravenously, similar to a blood transfusion. As donor cells circulate in the blood stream, they respond to cues that guide them to the bone marrow to establish a new hematopoietic, and subsequently, immune system. Blood counts — including white blood cells, red blood cells and platelets — fall in the days that follow due to the effects of the conditioning regimen. Transfusions of red blood cells and platelets are usually required. For white

blood cells, a medication called granulocyte-colony stimulating factor (G-CSF) to encourage efficient growth of new monocytes and neutrophils from the donor stem cells may be provided. Pain management for oral mucosal breakdown (mucositis) and nutritional support are usually required during this phase of HCT; however, with some RIC regimens used for DC, the severity of these symptoms is decreased.

In the subsequent weeks, patients are monitored closely for signs of complications such as infections, organ dysfunction, metabolic disturbances, vascular leak, and acute GVHD. Medications for GVHD and infection prophylaxis continue to be administered. The conditioning regimen can damage endothelial cells which line blood vessels throughout the body [41]. In some cases damaged endothelial cells accumulate, blocking blood flow through the liver causing veno-occlusive disease (VOD) [42]. Symptoms include abdominal pain, particularly in the location of the liver, weight gain/fluid retention, and jaundice (increased bilirubin on liver blood labs). Damaged endothelial cells can additionally block small blood vessels called capillaries in organs such as the kidneys, small bowel and lungs — consuming platelets, activating a portion of the immune system called complement, and shearing red blood cells trying to pass. This latter constellation is called transplant-associated thrombotic microangiopathy (TA-TMA) [43]. Signs/symptoms can include high blood pressure, anemia, thrombocytopenia, acute renal insufficiency, and mental status changes, among others. Both VOD and TA-TMA have targeted therapies should they be needed.

Neutrophil engraftment is defined as recovery of ANC to ≥ 500 cells/mm^3 for three days, and usually occurs between days 14 and 35 after graft infusion. Timing of neutrophil engraftment is associated with the stem cell donor source, cell dose, and occurrence of any complications that may delay hematopoiesis. Each cell type has a unique timeframe for development from stem cell differentiation to maturity and release into circulation. Because of these differences, red blood cell and platelet transfusion dependence may continue even after neutrophil engraftment. Unfortunately, some patients experience graft failure after HCT, either through active rejection of the donor cells by the patient's immune system or loss of the graft related to active infection or problems in the bone

marrow niche, or stem cell home [44]. Efforts to avoid graft failure include optimal HLA-matching, providing adequate stem cell dose, adequately targeting the patient's immune system during conditioning, and screening for circulating anti-HLA antibodies in the patient before HCT. Graft failure can occur early or late after transplant, with late or secondary graft failure arising after neutrophil engraftment had previously been demonstrated. In some cases, a patient's own stem cells can recover providing a safety net of hematopoiesis. However, in the majority, graft failure is accompanied by neutropenia and requires new or additional stem cells for recovery and survival.

Post-HCT Care

Patients are discharged from the hospital after neutrophil engraftment if: (1) there are no signs of infection or significant organ dysfunction, (2) they are able to maintain adequate hydration, nutrition and symptom control, and (3) an appropriate outpatient care management plan is in place. To reduce the risk of infections, patients are restricted from social contacts for 6-12 months after HCT, including work/school and participation in crowded indoor functions. The first 100 days after transplant are considered the highest risk time period for HCT-related complications. Patients may need to relocate temporarily to be in close proximity to a transplant center. Clinic visits are typically multiple times per week to administer medications and/or transfusions, and to assess for infection, graft function, GVHD, medication toxicity, metabolic derangements, and other post-HCT complications. If the patient is doing well after this period, the central venous catheter may be removed, and clinic visits may decrease in frequency. If the patient has traveled to a transplant center for HCT, care may be transitioned to providers closer to the patient's home, depending on several factors.

Immunosuppressive medications to prevent GVHD and infection prophylaxis are usually reduced or eliminated after 6-9 months, depending on the regimen, the patient's clinical status, and the transplant center's practice. In an ideal scenario, by one-year post-HCT, the patient will have discontinued almost all transplant-related medications, will be independent of transfusions, and resumed normal activities at home, school, or work. At

this time re-immunization can also start, as the transplant process will cause most patients to lose the protective effect of their previous immunizations.

The timing for discontinuation of immunosuppression to prevent GVHD, infectious prophylaxis, and re-immunization rely on knowledge of immune recovery after HCT [45]. Most lymphocytes live for a week to a few months. Therefore, donor lymphocytes present in the graft that may recognize the patient's body as foreign and cause GVHD, if adequately controlled, will have died by 6 months after HCT. This allows for discontinuation of immunosuppression. At the same time, the donor stem cells in the patient's bone marrow give rise to a new immune system, with specific cell populations recovering at different times following HCT [46]. Innate immune cells, early responders to infection and tissue damage, such as neutrophils and natural killer (NK) cells recover fastest, providing protection from some bacteria and fungi. Lymphocytes, including the B cells which produce protective antibodies and T cells that both instruct B cells and respond to innate immune signals to help eliminate infection or damaged/abnormal patient cells, are slower to recover. T and B cells are particularly important for response to viral infections. T cell development is particularly time consuming [47], as precursor T cells must travel from the bone marrow to an organ in the chest called the thymus. In the thymus, these developing T cells are educated on "self" versus "foreign" before being allowed to circulate to prevent autoimmunity. Many T cell populations take 9-12 months to reach normal numbers in circulation. Recovery timing may vary based on HCT conditioning regimen and immunosuppression approach to prevent or treat GVHD. Adequate response to immunizations requires both T and B cells, hence the typical re-immunization initiation between 6-12 months post-HCT [48]. Live-attenuated vaccines are often delayed until 2 years after HCT to prevent possible vaccine-mediated infection [49].

The transplant physician or hematologist coordinating the patient's care should continue comprehensive surveillance for DC-related complications in the immediate post-transplant period. Several reports have documented the overlap of chronic GVHD symptoms and non-hematological manifestations of DC [50-52], including oral mucosal,

skin, and hair changes, musculoskeletal abnormalities, and lung disease. In some cases of presumed idiopathic aplastic anemia, these manifestations have led to a diagnosis of DC in the months to years following HCT. Awareness and careful evaluation are required to discern between HCT-related complications that may require aggressive interventions such as corticosteroids, versus the natural progression of DC.

Long-Term Care

Optimal care of all patients who have undergone HCT requires lifelong regular and comprehensive evaluation; late effects of the conditioning agents and immunosuppressive medications used in HCT, and complications, such as GVHD and infections, demand ongoing surveillance. There is increased concern for significant post-HCT sequelae in patients with DC given the nature of the underlying disease. Notably, HCT only addresses the hematopoietic and immune complications of DC. All other cell types of the body remain with short telomere lengths and associated risks. While there are concerns that transplant toxicity may expedite complications such as pulmonary fibrosis or development of malignancies, data to support or refute such concerns are lacking. Certainly HCT would not be expected to improve pulmonary outcomes, and pulmonary complications remain a predominant cause of late post-HCT mortality [15, 51].

DC patients should undergo regular, comprehensive multi-disciplinary evaluations with appropriate targeted testing in the years following HCT [53]. Late effects of alkylating agents and radiation include malignancy, fertility problems, and endocrine defects, to which DC patients are predisposed. Chronic GVHD and prolonged use of corticosteroids or other immunosuppressive therapies may exacerbate bone disease and magnify risk of malignancy in DC. Lung complications of HCT may decrease pulmonary reserve and accelerate respiratory decline in these patients. HCT late effects and their overlap with DC are listed in Table 3.

Table 3. Overlap between manifestations of DC and HCT late effects.

	DC	HCT Late Effects
Hematology	Bone marrow failure, iron overload	Iron overload
Dermatology	Reticular pigmentation changes, skin thickening, nail changes	Chronic GVHD: rash, skin thickening and tightening, nail changes
Ophthalmology	Tear duct obstruction, loss of eyelashes	Ocular GVHD and dry eyes, cataracts
Oral	Leukoplakia, dental problems	Oral GVHD, dental problems
Endocrine	Skeletal defects, short stature, hypogonadism	Thyroid defects, growth hormone deficiency, fertility problems, hypogonadism, metabolic syndrome
Pulmonary	Fibrosis, arteriovenous malformations	Fibrosis, emphysema, pulmonary infections, idiopathic pneumonia syndrome, chronic GVHD
Gastroenterology	Esophageal stenosis, enteropathy, enterocolitis, cirrhosis, portal hypertension	Sequelae of gut GVHD, infectious colitis
Neurology, Psychiatry, Social	Development and psychiatric disorders, quality of life issues	Neurocognitive defects, post-traumatic stress disorder, anxiety, depression, social restrictions, quality of life issues
Oncology	MDS/leukemia, squamous cell cancers of head/neck/mucosal surfaces	Secondary MDS/leukemia, skin, and other cancers

With HCT survival improving for DC patients, deliberate attention must be given to coordinating and facilitating ongoing multi-disciplinary care, preventing long-term complications, and optimizing quality of life. Patient encounters should include

counseling on a healthy lifestyle and avoidance of harmful habits such as smoking and excessive alcohol consumption, which may accelerate lung and liver disease. Similarly, patients should avoid the DNA damage conferred by ultraviolet radiation of unprotected sun exposure on the skin already prone to squamous cell carcinoma development. Human papillomavirus vaccination is encouraged given the contributions of this virus to head and neck squamous cell carcinoma. Ideally, to anticipate problems and intervene appropriately, post-HCT and long-term care of DC patients should be coordinated by a provider or combination of providers knowledgeable about both DC-related complications and the late effects of HCT. At minimum, post-HCT DC patients should undergo annual pulmonary function testing, blood test of liver function, and comprehensive exams for cancerous or pre-cancerous lesions (complete skin exam by a dermatologist, oral/head/neck exams by dentistry and ENT, and anorectal/vaginal exams by an internist, urologist, and/or gynecologist). Vascular malformations of the lung, liver, or gastrointestinal tract may also be present and require attention.

Challenges and Opportunities

In 2022, through multi-center efforts, disease-specific approaches, and coordinated long-term multi-disciplinary care, improvements are being realized in HCT outcomes for patients with DC, demonstrating it to be an effective and feasible curative strategy for BMF. Ongoing challenges include tailoring HCT regimens for high-risk patients, such as those with allo-sensitization as they will have a higher risk of graft rejection, and those with significant DC-associated co-morbidities who may not tolerate RIC. As recognizing the diagnosis of an underlying TBD increases in adults with MDS and leukemia, who suffer high treatment-related mortality from conventional HCT approaches [54, 55], there is also a pressing need for pre-emptive strategies and/or trials of alternative conditioning agents. To this end, in the last several years, gene therapy has advanced in several non-malignant conditions. Genetic modification, replacement or repair of blood cells using viral transduction, CRISPR/Cas9 and/or base editing can be seen on the horizon for DC. The successful demonstration of safety and efficacy using autologous

gene therapy would drive pre-emptive strategies to prevent MDS/leukemia in DC, albeit with the risk that residual uncorrected cells might transform. Advances will also be needed to reach organs and tissues other than the blood affected in DC patients. Alongside genetic approaches, novel conditioning agents such as antibodies against CD45 or CD117 are being developed to avoid non-targeted cellular cytotoxicity, which could be of particular benefit in DC/TBD patients. One can expect trials of all of the above strategies in the coming years, in hopes of fundamentally changing the experience and outcomes of hematologic complications in DC/TBD.

Acknowledgement

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Chapter 14

Pulmonary Fibrosis

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Introduction

Pulmonary fibrosis is one of the most serious and life-threatening complications of the telomere biology disorders (TBDs). Pulmonary fibrosis represents a heterogeneous group of conditions termed the fibrotic Interstitial Lung Diseases (ILDs), which are characterized by the deposition of collagen and extracellular matrix in the space between alveolar epithelial cells and capillary endothelial cells.

Pulmonary fibrosis can manifest across the age spectrum in patients with TBDs. Pulmonary fibrosis in young patients with dyskeratosis congenita (DC) has been

described after hematopoietic cell transplantation (HCT) for bone marrow failure. Pulmonary fibrosis in this setting may be accelerated by exposure to conditioning regimens for hematopoietic cell transplantation [1, 2]. In patients who have received a HCT, respiratory symptoms develop early in life (median 14 years), with survival to early adulthood [3]. Pulmonary fibrosis may also occur in patients with DC later in life, in the absence of HCT, and may also be found concurrently with cytopenias [4, 5]. For this group of DC/TBD patients, respiratory symptoms develop later (median 37 years), and median survival is longer. Finally, pulmonary fibrosis may be the dominant, and only, clinical manifestation of telomere-mediated disease [6-9]. Patients presenting in this manner are typically older and do not have the mucocutaneous findings or severe bone marrow failure associated with DC, although they may have a family or personal history of less severe DC-associated phenotypes. The most common diagnosis for this last group of patients is Idiopathic Pulmonary Fibrosis (IPF), which is typically diagnosed after the fifth decade of life [10]. Regardless of when the pulmonary fibrosis starts, it is usually relentlessly progressive and leads to respiratory failure. Considering that the prevalence of IPF associated with TBDs is estimated to be greater than the prevalence of classic DC, IPF is recognized as one of the most common TBD presentations [11].

Clinical Presentation

Patients typically present with respiratory complaints including exertional dyspnea (shortness of breath) and chronic cough. They may have inspiratory rales and digital clubbing on physical exam. The disease is associated with a restrictive pattern on

pulmonary function testing and decreased diffusion capacity for carbon monoxide (DL_{CO}). Screening chest X-rays may appear normal during the early stages of disease, which is why high-resolution computed tomography (HRCT) imaging of the chest is the gold-standard diagnostic study. HRCT often demonstrates diffuse interstitial markings (reticulations), architectural distortion of the airways (traction bronchiectasis), and loss of normal lung parenchyma in scarred tissue (cysts, honeycombing).

The pattern of lung involvement is often complex in patients with DC and pulmonary fibrosis. Lung histopathology generally features a mixture of cellular inflammatory infiltrates and interstitial fibrosis that does not typically mirror the findings in older adults. Assessing these patients may be particularly difficult not only because the clinical findings and histopathology are non-specific, but because of the range of possible differential diagnoses, including lung involvement of graft versus host disease after HCT, opportunistic infection, and drug-induced lung injury.
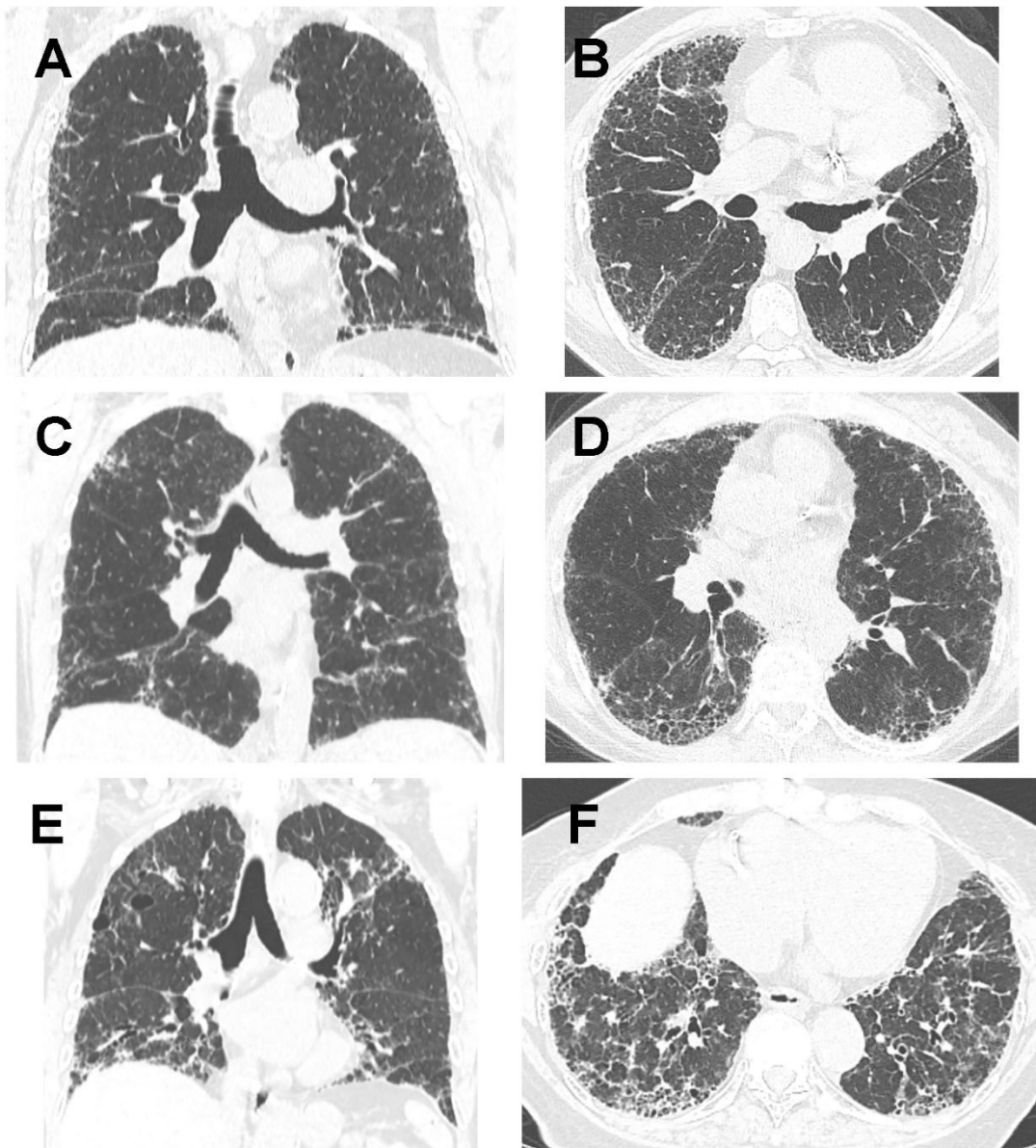
Figure. Representative high-resolution computed tomography (HRCT) chest images from adult pulmonary fibrosis patients with rare, deleterious variants in telomere related genes. A 78 year old man heterozygous for *TERT* p.Cys76Stop (**A, B**), a 55 year old man heterozygous for *PARN* c.1222+1G>T (**C, D**), and an 81 year old female heterozygous for *RTEL1* p.Ile110frameshift (**E, F**). Coronal images at the level of the carina are shown in **A, C,** and **E**. Axial images are shown in **B, D.** and **F**.

Guidelines for providing an accurate ILD diagnosis in adults have evolved over the last decade [12-14]. As with any chronic lung disease, a thorough medical history is

necessary to determine if there are underlying environmental insults or comorbidities that may be contributing to the lung disease. In certain clinical contexts, when there is no clear cause of the pulmonary fibrosis, a diagnosis of IPF is considered. This diagnosis requires a definite or probable radiographic pattern of usual interstitial pneumonitis (UIP) on HRCT. In cases in which the radiographic pattern is indeterminate or not consistent with UIP, evaluation of lung tissue is often needed to make a definitive diagnosis. However, the risks and benefits of a surgical lung biopsy should be carefully weighed, as surgical biopsy has been associated with increased mortality in patients with TBDs [15], and no significant difference in survival has been found in patients with different fibrotic ILD diagnoses [10]. Thus, clinical work-up, including the least invasive procedures, and multidisciplinary discussion are recommended.

Telomere-Related Genetic Variants Associated With Pulmonary Fibrosis

Rare, damaging, protein-coding variants in several telomere-related genes linked to DC are enriched in patients with fibrotic ILDs (see also Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders and Chapter 5, Genetic Counseling for Families). In older adults, they are found most (~25%) in patients with a family history of pulmonary fibrosis (FPF) and less commonly (~5%) in those with sporadic IPF [16]. Pathogenic variants in the telomerase genes (*TERT, TERC*) are most commonly represented [6, 7, 17], followed by variants in *PARN* and *RTEL1* [18-22]. Fewer FPF kindreds and cases have been described with pathogenic variants in *NAF1* [23], *DKC1* [24, 25], *NHP2* [26], *TINF2* [27-29], *NOP10* [30, 31] and *ZCCHC8* [32].

Individuals with deleterious variants in telomere biology genes have evidence of short telomeres (see also Chapter 3, Diagnosing TBDs). The manifestations of TBDs follow the general trend that affected pediatric patients have mean lymphocyte telomere lengths far below the 1st percentile for their age, those presenting in early adulthood have telomere lengths <1st percentile, and patients >50 years of age have more modest telomere shortening, i.e., <10th percentile for their age [33]. When evaluating for short telomeres in individuals with rare variants in telomere-related genes, a cutoff of mean lymphocyte telomere length <1st percentile by flow-FISH (see Chapter 3, Diagnosing TBDs) is usually employed to implicate a diagnosis of DC [34]. The appropriate cutoff for adults is less well established.

Fibrotic ILD Associated With Short Telomeres and Telomere Biology Gene Pathogenic Variants

Heterozygous rare, deleterious, genetic variants in telomere biology genes have been linked with different clinical ILD diagnoses that can lead to progressive forms of pulmonary fibrosis [10]. For adults, a clinical diagnosis of IPF is typically the most common, accounting for about 50% of cases [10]. Unclassifiable ILD, chronic hypersensitivity pneumonitis (CHP), connective tissue disease-associated ILD (CTD-ILD) pleuroparenchymal fibroelastosis, and other idiopathic interstitial pneumonias make up the other half of cases [10, 35]. Extra-pulmonary manifestations, including macrocytosis, thrombocytopenia, liver disease, and cutaneous abnormalities, may be prevalent in carriers of rare genetic variants [8, 17].

Age at the time of pulmonary fibrosis diagnosis correlates with gene mutation and degree of telomere shortening. DC/TBD patients with *DKC1, NHP2,* or *TINF2* mutations have a younger age of ILD onset than those with *TERT* or *TERC* mutations [15]. For adult-onset pulmonary fibrosis, patients with *TERC* mutations are diagnosed with a fibrotic ILD at an earlier age (mean 51 years), than those with *TERT* (58 years), *RTEL1* (60 years), or *PARN* (65 years) mutations [10].

Fibrotic ILD Associated With Short Telomeres, With No Identifiable Telomere Biology Gene Pathogenic Variant(s)

The telomere length cutoff considered to be "short" is not well established for adults with pulmonary fibrosis. Age-adjusted peripheral blood leukocyte telomere length <10th percentile is frequently seen in patients with FPF and sporadic IPF without identifiable telomere-related mutations [36, 37]. There are now at least 12 independent IPF cohorts across the globe that demonstrate evidence of telomere shortening of this degree [17, 19, 37-43]. The percentage of patients with various non-IPF fibrotic ILDs, such as CHP [44], unclassifiable ILD [45], rheumatoid arthritis-associated ILD [46], and other CTD-ILDs [47], with age-adjusted telomere length <10th percentile is higher than would be predicted, but to a lesser degree than what is observed for IPF. Mendelian randomization studies suggest that telomere length, identified from a polygenic risk score, is causally related to the development of IPF, but not COPD, in the UK Biobank [48]. Thus, short telomeres are a common finding in, and are likely causally related, to a wide array of fibrotic ILDs.

The explanation for short telomeres in patients with no identifiable rare genetic mutation in a telomere biology gene is unclear. Combinatorial effects from common genetic variants associated with short telomeres may explain some proportion of patients [49, 50]. Environmental factors, such as cigarette smoking, may contribute [51]. Additionally, epigenetic inheritance of short telomeres may contribute to this heritability gap. Family members of telomere biology gene variant carriers with pulmonary fibrosis, who did not inherit the mutation themselves, may harbor short telomeres [52].

There is an inverse association between telomere length and lung transplant-free survival for patients with IPF [19, 38-40, 42], CHP [53], and interstitial fibrosis with autoimmune features (IPAF) [46], independent of patient age, sex, ethnicity, and baseline Forced Vital Capacity (FVC) and diffusing capacity of the lungs for carbon monoxide (DL_{CO}). Similarly, the rate of FVC decline is faster for IPF, CTD-ILD, and IPAF patients with leukocyte telomere length <10th percentile versus those with ≥10th percentile [46]. Thus, telomere length is a biomarker that can inform clinically relevant outcomes for adults with a variety of fibrotic ILDs.

Medical Treatment

Immunosuppression poses increased risk of adverse outcomes in patients with IPF, particularly in those with short telomeres [15, 43]. Similarly, in patients with CHP, immunosuppression shows no efficacy in those with the shortest telomere lengths [54]. As such, patients with pulmonary fibrosis and short telomeres should be treated with

immunosuppressive therapies only when benefits outweigh risks, such as after lung transplantation, and should be carefully monitored for infectious complications.

One phase 1-2 clinical trial showed that danazol, a synthetic sex hormone with androgenic properties, was associated with telomere elongation and hematologic response in some patients with TBDs and pancytopenia [55]. The effect of danazol in slowing pulmonary fibrosis is currently unknown but is undergoing study in ongoing clinical trials.

Clinical trials that have led to FDA approval of pirfenidone [56] and nintedanib [57] as antifibrotic therapies for IPF have not enrolled or stratified patients by telomere length. These studies have included large numbers of patients and have shown that the rate of FVC decline was significantly lower among patients who received an antifibrotic than among those who received placebo. Meta-analysis of ~13,000 patients with IPF across 26 studies have shown improved survival and fewer acute exacerbations in those patients taking these antifibrotics [58]. Lower risk of all-cause mortality and hospitalization of patients with IPF taking antifibrotics as compared to patients with no treatment have also been seen by analyzing large US insurance databases [59]. Recently, nintedanib was also FDA approved for progressive fibrosing ILD, based on a double-blind, placebo-controlled, phase 3, international clinical trial [60].

Only a handful of studies have evaluated treatment of TBD-mediated pulmonary fibrosis with antifibrotic medications. Post-hoc analysis of two phase 3 clinical trials indicates a reduced rate of FVC decline in IPF patients with short telomeres randomized to

treatment with pirfenidone as compared to placebo [19]. Safety and efficacy of the antifibrotics for IPF patients who carry a telomere biology gene pathogenic variant have been reported [61].

Thus, it is our recommendation that TBD patients with IPF or progressive pulmonary fibrosis should be started on an antifibrotic medication. Those with interstitial lung abnormalities (ILA) in a non-fibrotic or a non-UIP pattern should be followed with serial pulmonary function tests annually or more frequently depending on symptom progression. Repeat HRCT scans can be performed if there are progressive symptoms or pulmonary function test (PFT) decline to determine if there is progressive pulmonary fibrosis.

Screening for Pulmonary Fibrosis

There are few studies assessing the utility of screening protocols for pulmonary fibrosis in DC/TBD. Some providers feel that chest imaging poses too high a risk from medical radiation for children relative to its potential benefit. Pulmonary function testing affords no exposure to radiation, and thus, is safer means of determining functional limitations. Given the risk of pulmonary complications after HCT, all patients should have careful assessment of lung function prior to HCT. Additionally, current consensus guidelines suggest lung function tests every 3 months for 2 years following HCT [62]. For individuals with persistently diminished lung function, further work-up with imaging and bronchoscopy should be considered.

Asymptomatic carriers of telomere biology gene pathogenic variants have a very high prevalence of pulmonary fibrosis, which increases with age. ILAs, which are subtle and often incidentally found, are thought to represent early ILD in high-risk individuals [63]. In one study, fifty percent of at-risk family members with rare *TERT* variants were found to have ILA and a DL_{CO} less than 80% predicted [64]. Similarly, adults with just a family history of fibrotic lung disease are at higher risk for pulmonary fibrosis. The estimated prevalence of early or subclinical manifestations of pulmonary fibrosis in relatives of individuals with familial pulmonary fibrosis ranges from 15-22% [65, 66]. Development of ILA in family members of patients affected with sporadic IPF or pulmonary fibrosis due to other etiologies is dependent on the presence of environmental risk factors (such as cigarette smoking) and common genetic variants, including the *MUC5B* promoter risk allele (rs35705950) [67].

Given that the FDA approved therapies for fibrotic ILD are not curative and do not reverse fibrosis, their utility in slowing down the rate of progression is best if implemented early in the course of disease. Thus, for family members at high risk of disease (such as mutation carriers or those with a strong family history of disease), we recommend a screening HRCT scan of the chest, spirometry, and plethysmography 10-15 years before the earliest manifestation of ILD in the family. The age at which to start screening should consider effects of genetic anticipation related to accelerated telomere shortening.

In symptomatic individuals with a family history of pulmonary fibrosis and/or evidence of a personal or family history of a TBD (such as early graying of hair before 30 years of

age, idiopathic liver disease, cytopenia, macrocytosis), we recommend telomere length testing as part of the workup [68]. We recommend genetic testing for inherited mutations if the peripheral blood leukocyte telomere length falls below the 10th percentile, with cascade testing of pathogenic or likely pathogenic variants in at-risk family members. Individuals with a family history of pulmonary fibrosis without evidence of a TBD may wish to undergo genetic testing, but the likelihood of discovering pathogenic or likely pathogenic variants is typically low, especially if there are few affected individuals in the kindred. Currently, we do not recommend telomere length testing in individuals with sporadic pulmonary fibrosis without a personal or family history suggestive of a TBD.

Exposures to Avoid

The development of pulmonary fibrosis is associated with various environmental, occupational, and iatrogenic exposures. Vigilance is needed to avoid these insults, especially for those that have a genetically inherited susceptibility to ILD. The following list, although not comprehensive, includes:

- **Smoking.** Cigarette smoking is known to accelerate the onset of lung disease and is associated with various ILDs [69]. Smoking of cigarettes, cigars, pipes, e-cigarettes, vaping, hookahs, and recreational drugs all lead to lung injury and increased risk of ILD. Smoking should be strongly discouraged, and multi-disciplinary effort should be made to support patients in avoiding both

primary and secondary sources of smoke. Referral to support groups, counseling, and medication aides should be considered in high-risk populations.

- **Cytotoxic medications and radiation.** Ionizing radiation should be minimized and procedures for aggressive lung shielding should be implemented [70]. Cytotoxic medications used as conditioning agents prior to HCT should be avoided whenever possible [1, 2]. Preparative agents with the smallest potential for pulmonary toxicity should be considered.

- **Medications.** Several medications are strongly associated with pulmonary toxicity [71], such as amiodarone [72] and nitrofurantoin [73]. A growing number of checkpoint inhibitors are associated with increased incidence of ILD. Some anti-depressants are associated with increased risk of ILD in older adults [74]. These medications should be avoided when possible.

- **Surgical risk.** Exacerbations of lung disease in adults with ILD have been well-documented following both pulmonary and non-pulmonary surgeries. The risk should be weighed in planning elective procedures because these complications can be fatal. Pirfenidone has been shown to be safe and promising for reducing the risk of acute exacerbations of IPF in patients undergoing lung cancer surgery [75] but has not been studied in patients with TBD. When feasible, elective surgery is preferably pursued using regional anesthesia to avoid aspiration or high partial pressure oxygen, which can cause alveolar epithelial injury.

- **Occupational and environmental risk factors.** Occupations and exposures that have been associated with an increased risk of ILA progression in individuals at risk for familial ILD include aluminum smelting as well as lead, bird, and mold exposure [76]. Exposure to a number of organic antigens (most commonly bird feathers, fungal, and bacterial antigens) can result in chronic hypersensitivity pneumonitis (CHP), which can mimic IPF and other fibrosing ILDs. Changing occupations is not feasible for many individuals. In these cases, implementing respiratory protection plans that include wearing a particulate-filtering respiratory may reduce hazards associated with these exposures.
- **Respiratory illness.** Infections suspected or confirmed to be caused by bacterial pathogens should be promptly and appropriately treated with antibiotics. Immunizations to respiratory tract pathogens should be offered.

Lung Transplantation

Lung transplantation is the only known modality that cures fibrotic ILD. Please refer to Chapter 15, Lung Transplantation for more details.

Conclusion

Pulmonary fibrosis is one of the most common and life-threatening complications of the TBDs. Treatment with antifibrotic agents offers promise for patients with IPF or progressive pulmonary fibrosis in slowing the rate of respiratory decline, but current medications do not halt or reverse the disease. Additional studies are needed to

specifically study the effects of antifibrotic medications in patients with TBDs. Thus, screening for pulmonary fibrosis in high-risk individuals, avoidance of environmental contributors to fibrosis, and consideration of early implementation of antifibrotic treatment should be cornerstones of clinical management.

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Chapter 15

Lung Transplantation

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Introduction

Lung transplantation is the only definitive treatment for end-stage interstitial lung disease (ILD). Experience with lung transplantation for patients with classic DC is limited, primarily consisting of case reports in individuals who have previously undergone hematopoietic cell transplant (HCT) [1]. As with lung transplantation following HCT for other causes (e.g., pulmonary graft-versus host disease), careful selection of affected individuals including consideration of infectious risk and other HCT or DC-related organ dysfunction is necessary to achieve successful outcomes [2].

There is, however, a growing literature regarding lung transplantation among adults with short telomere-related ILD. Individuals with ILD and short telomeres with or without known telomere biology disorder (TBD) due to pathogenic germline variants in TBD-associated genes are at risk for more rapid disease progression and decreased transplant-free survival compared to those with normal telomere lengths [3, 4]. Patients and providers should consider early referral to a lung transplant center to begin the evaluation process. Even for individuals who are currently too well for listing, having completed the transplant evaluation can create a safety net in the event of rapid disease progression requiring urgent listing.
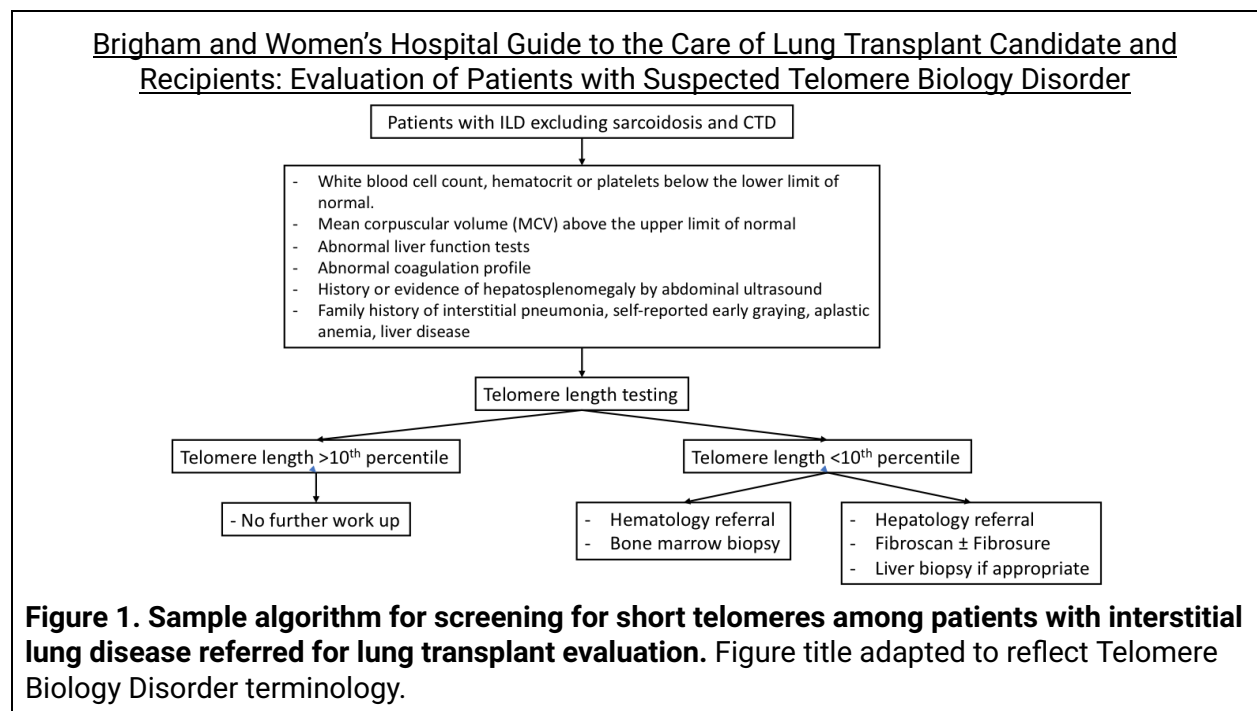
Transplant Evaluation

Despite a growing awareness of the role of short telomeres in pulmonary fibrosis, the majority of patients with ILD who are referred to a lung transplant center will not have been screened for short telomeres. We recommended screening for telomere length in individuals with a personal history of early graying (before 30 years old), cytopenias (low blood cell counts) or macrocytosis (large red blood cells), and/or abnormal liver function tests or imaging suggestive of hepatic impairment without other explanation; or with a family history of one or more first degree relatives with ILD. Figure 1 and Figure 2 illustrate two potential screening protocols. Additional assessment, including evaluation for TBD-related mutations, should occur in close collaboration with medical genetics clinicians and genetic counselors.
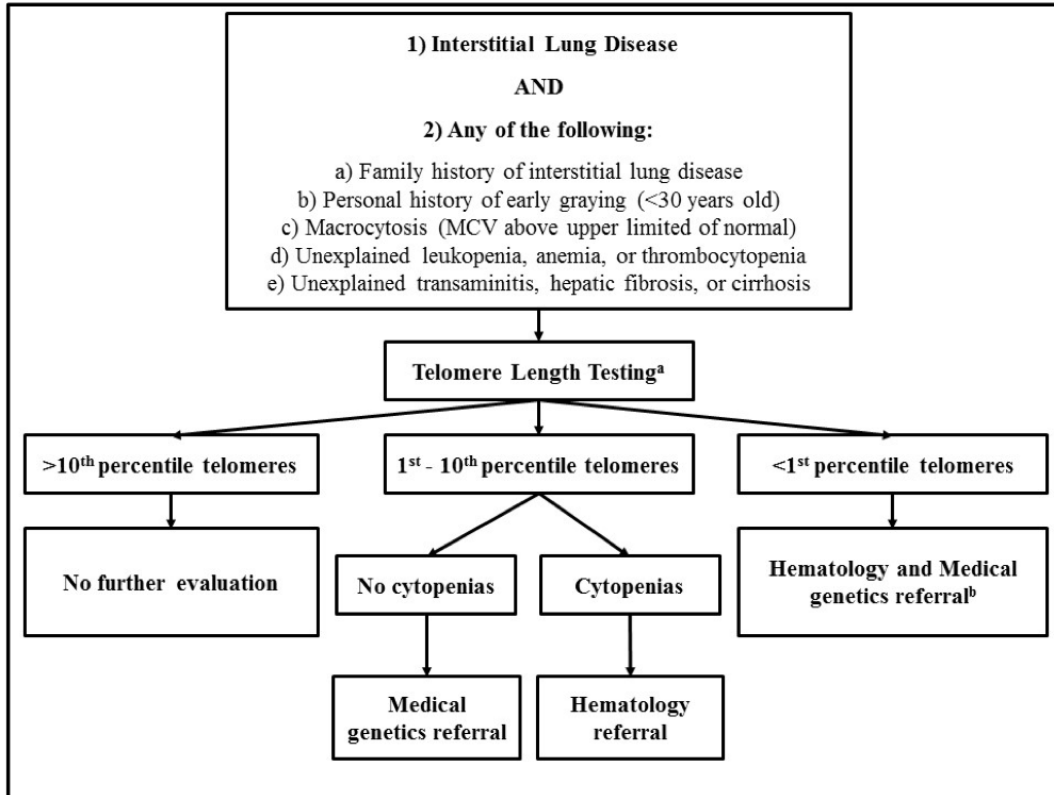
Importantly, the goal of telomere length screening is not to identify a contraindication to lung transplantation. In our opinion, it is important to identify candidates with short telomeres so as to stratify their risk for extra-pulmonary disease manifestations and to design appropriate post-transplant management strategies to allow successful

transplantation. For example, early case series suggested that lung transplant recipients with short telomeres were more likely to have hematologic complications [5, 6]. Because of the risk of bone marrow failure, two authors (SEC and DH), recommend routine bone marrow biopsy as part of the lung transplant evaluation for all patients with short telomeres (<10th percentile for age) [6] (Figure 1). Other programs, however, only proceed with bone marrow biopsy in the presence of significant cytopenias in one or more cell lines. For potential candidates with severe hypocellular bone marrow without malignant transformation, consideration should be given to referral to a center that can offer tandem lung and bone marrow transplant [7]. Two authors (SEC and DH) recommend routine liver imaging such as FibroScan to evaluate for cryptogenic fibrosis or cirrhosis in all candidates with short telomeres. We do not, however, recommend routine liver biopsy as part of the transplant evaluation in the absence of imaging or biomarkers suggestive of hepatic dysfunction [6]. For potential candidates with hepatic fibrosis and elevated portal pressures or with cirrhosis, we recommend evaluation for combined lung-liver transplantation, when appropriate [8].

Brigham and Women's Hospital Guide to the Care of Lung Transplant Candidate and Recipients: Evaluation of Patients with Suspected Telomere Biology Disorder

Patients with ILD excluding sarcoidosis and CTD

- White blood cell count, hematocrit or platelets below the lower limit of normal.
- Mean corpuscular volume (MCV) above the upper limit of normal
- Abnormal liver function tests
- Abnormal coagulation profile
- History or evidence of hepatosplenomegaly by abdominal ultrasound
- Family history of interstitial pneumonia, self-reported early graying, aplastic anemia, liver disease

Telomere length testing

Telomere length >10th percentile

- No further work up

Telomere length <10th percentile

- Hematology referral
- Bone marrow biopsy

- Hepatology referral
- Fibroscan ± Fibrosure
- Liver biopsy if appropriate

Figure 1. Sample algorithm for screening for short telomeres among patients with interstitial lung disease referred for lung transplant evaluation. Figure title adapted to reflect Telomere Biology Disorder terminology.

Hospital of the University of Pennsylvania Lung Transplant Program Guidelines:
Approach to Evaluation of Suspected Telomere Biology Disorder

Screening Algorithm:

1) Interstitial Lung Disease

AND

2) Any of the following:

a) Family history of interstitial lung disease
b) Personal history of early graying (<30 years old)
c) Macrocytosis (MCV above upper limited of normal)
d) Unexplained leukopenia, anemia, or thrombocytopenia
e) Unexplained transaminitis, hepatic fibrosis, or cirrhosis

Telomere Length Testing[a]

>10th percentile telomeres

1st - 10th percentile telomeres

<1st percentile telomeres

No further evaluation

No cytopenias

Cytopenias

Hematology and Medical genetics referral[b]

Medical genetics referral

Hematology referral

[a] Screening by Flow-FISH
[b] Hepatology referral is warranted for individuals with liver imaging concerning for fibrosis or cirrhosis with or without evidence of portal hypertension

Figure 2. Sample algorithm for screening for short telomeres among patients with interstitial lung disease referred for lung transplant evaluation. Figure title adapted to reflect Telomere Biology Disorder terminology.

Transplant Outcomes

Although there is a growing literature on transplant outcomes among recipients with TBDs, differences in sample size, institutional management and immunosuppression protocols, and telomere length measurement assays make comparisons between reports difficult.

Several moderately-sized cohort studies have identified an association between short telomeres and increased post-transplant mortality and/or chronic lung allograft dysfunction (CLAD) [9-11]. For example, Newton et al found that recipients with ILD and telomeres below the 10th percentile had a 6-fold increased hazard for CLAD and a 10-fold increase hazard for death [10]. Swaminathan et al similarly reported higher mortality and CLAD among pulmonary fibrosis recipients with variants in *TERT*, *RTEL1*, and *PARN* [9]. More broadly, Courtwright et al found an association between decreased CLAD-free survival and shorter telomere length after transplant among all disease types, including cystic fibrosis and chronic obstructive pulmonary disease [12]. Importantly, however, despite the relative increase risk for mortality, overall survival for recipients with short telomeres in these studies has been in keeping with national benchmarks. In addition, not every study has shown an association between short telomeres and poor survival. For example, Faust et al did not find decreased CLAD-free mortality among short telomere recipients [13].

Even if the link between short telomeres and increased post-transplant mortality and/or CLAD is borne out in larger studies, the mechanisms behind this association remain unclear. It may be that recipients with short telomeres require immunosuppression reduction because of cytopenias, placing them at risk for CLAD. Alternatively, they may be more vulnerable to respiratory viral and other infections that are associated with CLAD, they may lack the replicative reserve to populate the donor organs with recipient-derived stem cells, or they may be more susceptible to fibroblast rather than epithelial proliferation following airway injury [14, 15].

Several other post-lung transplant outcomes aside from survival and chronic rejection have been reported in short telomere recipients. Popescu et al. identified impaired cytomegalovirus (CMV) immunity among patients with short telomeres and pulmonary fibrosis who underwent lung transplantation [16]. CMV reactivation was particularly common in mismatch recipients (CMV donor positive, recipient negative), which has been reported in other cohort studies in the short telomere lung transplant population [12]. There have also been case reports of bone marrow failure syndromes following

lung transplant, particularly for *TERT* variant carriers, as well as systemic graft-versus-host disease [5, 17]. Short telomere length, however, has not been associated with *de novo* donor specific antibody production or the development of more severe chronic kidney disease following transplant [11, 18]. There are also mixed associations between severe primary graft dysfunction and short telomeres [9, 10]. Some, but not all studies, have suggested decreased risk for acute cellular rejection—potentially related to impaired cellular immunity—among recipients with short telomeres [10, 11, 19].

Post-Transplant Management

While acknowledging the limitations of the current literature on post-transplant outcomes, we believe that there are steps that can be taken to optimize care pathways for lung transplant recipients with shortened telomeres. First, particularly for recipients with known hematologic manifestations, we recommend avoiding T cell depleting agents such as anti-thymocyte globulin (ATG), unless there is a strong clinical indication. ATG has been associated with increased telomere shortening and decreased telomerase activity in kidney transplant recipients and increases the risk of infectious complications following transplant [20]. Although a small case series did not show increased mortality among short telomere recipients with the use of the CD52 monoclonal antibody alemtuzumab, there was an increased incidence of neutropenia, thrombocytopenia, and need for red blood cell transfusion [21].

Second, given the apparent increased risk for CMV reactivation, we recommend lifelong CMV prophylaxis for recipients with TBDs, particularly among those who are CMV mismatches. Because valganciclovir, the most common CMV prophylaxis agent, is associated with bone marrow suppression, consideration should be given to alternative drugs such as letermovir. For CMV negative candidates, we do not recommend delaying lung transplant in favor of a CMV negative donor match given the potential for increased waitlist mortality. Finally, screening for post-lung transplant skin cancers is particularly

important in transplant recipients with TBDs, who are at high risk for these conditions (see also Chapter 6, Dermatologic Manifestations and Chapter 9, Solid Tumors) [22]. Correspondingly, the use of antifungal agents such as posaconazole or isavuconazonium should be considered, when indicated, rather than voriconazole given its association with skin cancer.

In the absence of clinical studies demonstrating post-lung transplant benefit, we do not recommend the routine use of danazol for lung transplant recipients with TBD-related mutations and refractory bone marrow suppression, including the pediatric population. Of particular concern is the potential for hepatic toxicity and venous thromboembolism in a population already at higher risk for these complications [23]. Similarly, although *in vitro* data have suggested that mammalian target of rapamycin (mTOR) may be associated with reduced telomere shortening compared to calcineurin inhibitors, we do not recommend routine use of mTOR inhibitors for TBD lung transplant recipients in the absence of another indication (e.g., chronic kidney disease, airway stenosis, etc.).

Conclusion

DC and the related TBDs are not a contraindication for lung transplantation, although early referral for lung transplant evaluations at an experienced center is warranted. Additional testing may be required to identify modifiable risks to tailor post-lung transplant management to achieve the best possible outcomes. When the evaluation identifies two-organ dysfunctions (lung-liver, lung-bone marrow), referral to specialized transplant centers for evaluation for dual transplantation is warranted.

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Chapter 16

Vascular Complications

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Introduction

The development of gastrointestinal (GI), pulmonary, and retinal vascular telangiectasias have recently been recognized as important complications associated with dyskeratosis congenita (DC) [1-3]. How vascular malformations are related to defective telomere biology is still not clear and further studies are warranted. Several biological mechanisms are currently discussed such as a connection between short telomeres and impaired wound healing, possibly leading to vascular dysfunction [2, 4, 5]. It is prudent to note that there may be other organs whose vascular involvement in DC and related telomere biology disorders (TBDs) have not yet been characterized.

Definitions	
Vascular	Refers to blood vessels, both arteries and veins.
Artery	Carries oxygenated blood from the heart to the rest of the body.
Vein	Carries oxygen-depleted blood toward the heart to then go to the lungs.
Capillary	Small blood vessels that connect arteries and veins.
Telangiectasia	A condition characterized by dilation of the capillaries, which causes them to appear as small red or purple clusters, often spidery in appearance, on the skin or the surface of an organ. Telangiectasias can be very delicate and bleed when disturbed.
Arteriovenous malformation	An abnormal tangle of blood vessels connecting arteries and veins, which disrupts normal blood flow and oxygen circulation.
Retinopathy	Damage to the retina of the eyes, which may cause vision impairment.

Retinal Telangiectasias

Coats plus is a rare phenotypically complex disorder that encompasses bilateral exudative retinopathy, retinal telangiectasias (Figure 1), intrauterine growth retardation (IUGR), intracranial calcifications, bone abnormalities with poor healing, and gastrointestinal vascular telangiectasias [6, 7] (see Chapter 3, Diagnosing Telomere Biology Disorders). The majority of Coats plus patients have biallelic variants in *CTC1*, a telomere capping gene. Coats plus was recognized as a DC-related telomere biology disorder when *CTC1* variants were discovered to also cause DC. Notably, some features of Coats plus, such as bilateral exudative retinopathy and intracranial calcifications, overlap with those of Revesz syndrome, another variant of DC (see Chapter 3, Diagnosing Telomere Biology Disorders) [6-8]. Therefore, individuals with DC should be regularly screened by a trained ophthalmologist for presence of retinal pathology such as retinal telangiectasias.
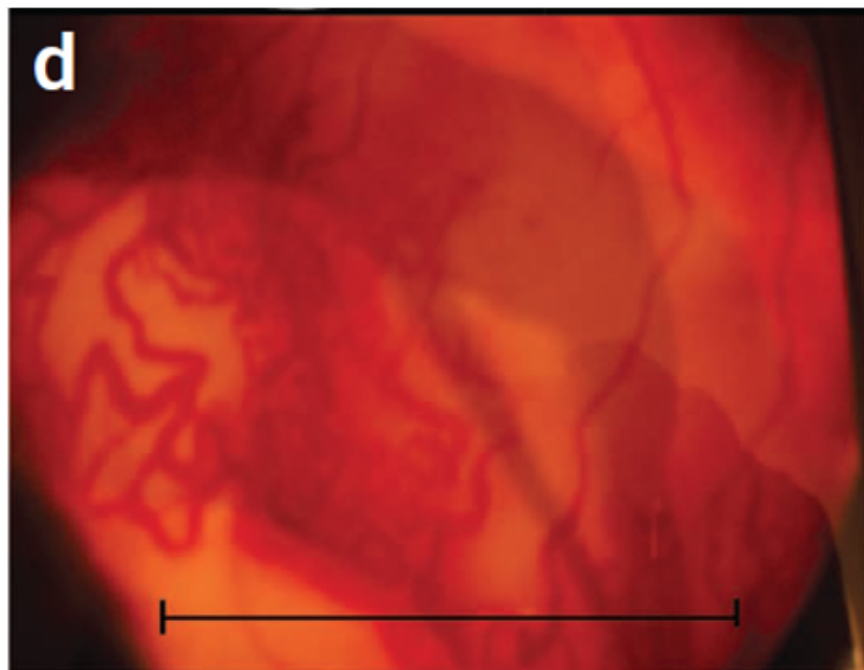
Figure 1. Retinal microangiopathy in Coats plus syndrome. Figure from Anderson BH, Kasher PR, Mayer J, et al. Mutations in *CTC1*, encoding conserved telomere maintenance component 1, cause Coats plus. *Nature genetics*. 2012;44(3):338-342.

Gastrointestinal Telangiectasias

GI telangiectasias (Figure 2) in DC may present as life-threatening GI bleeding and are thus a potentially severe complication of DC [2, 9]. Of note, in some cases of severe GI bleeding in DC patients no origin can be identified [2]. Vascular complications and severe GI bleeding from hemorrhagic colitis have been reported in post-HCT DC patients, suggesting that these patients are particularly vulnerable to GI-related morbidity and mortality [10-12]. In a recent review of the NCI DC cohort study participants, approximately 8% of transplanted patients were diagnosed with GI telangiectasias [13]. DC patients can also develop portal hypertension either from noncirrhotic liver disease or hepatic fibrosis, leading to development of porto-systemic varices [14].
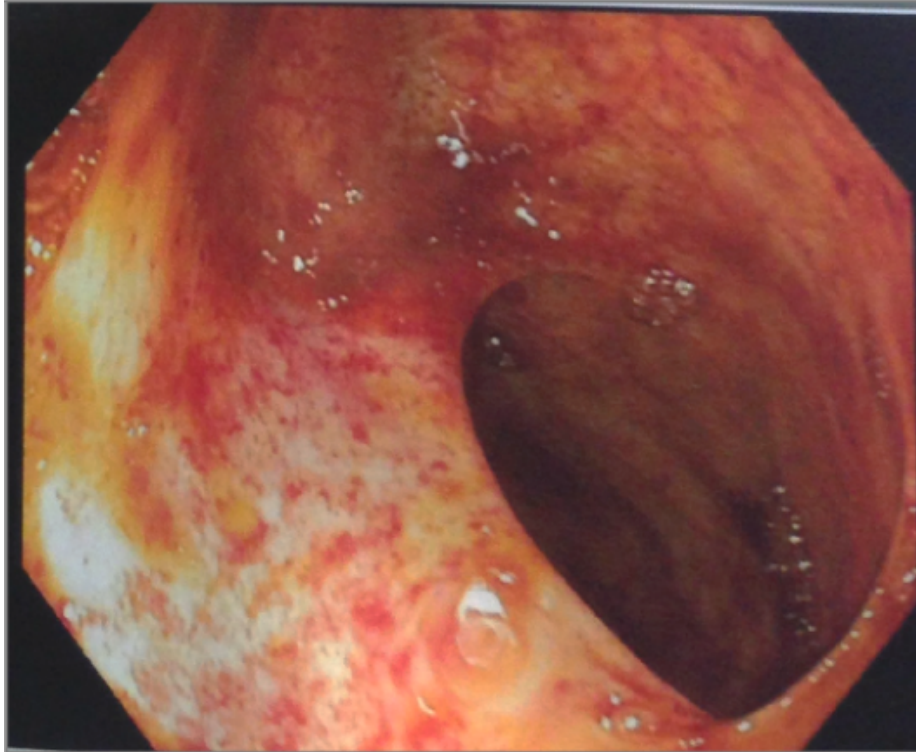
Figure 2. Telangiectasias of the small bowel lumen. Figure from Niewisch MR and Savage SA. An update on the biology and management of dyskeratosis congenita and related telomere biology disorders. *Expert Rev Hematol*. 2019 Dec;12(12):1037-1052.

Recent data indicate that bleeding GI telangiectasias may affect a broader group of individuals with TBDs than previously recognized [9]. In addition to individuals with Coats plus, which was expected, patients with dyskeratosis congenita, Revesz, and Hoyeraal-Hreidarsson syndrome were represented in the case series. The median age at the time of initial bleed was 12.5 years, but the range was wide, encompassing patients <1 to 36 years of age. Initial GI bleeding episodes were recognized both before and after HCT, suggesting that this complication represents the natural history of the disease in certain individuals, rather than an outcome of treatment. Most GI telangiectasias were located in the stomach and small bowel, with a minority of patients exhibiting these lesions in the large bowel (colon). No individuals in this series died as a result of an initial GI bleed, but recurrence of GI bleeding was almost universal (15/16 patients), and thus repeated hospitalizations and multiple diagnostic and therapeutic procedures highlight substantial morbidity associated with this manifestation of TBDs.

Diagnostic modalities for GI telangiectasia include upper GI endoscopy, capsule endoscopy, or colonoscopy. Liver ultrasound and computed tomography (CT) scans may indicate the presence of liver disease or fibrosis that can potentially cause portal hypertension, and clinicians must be particularly vigilant about development of varices in these patients.

Management

The optimal treatment strategy for bleeding related to GI telangiectasias remains unsettled. Most individuals are treated with supportive therapies in-hospital including transfusions of blood products, gastric acid-reducing medications, agents which coat/protect the GI tract, and drugs to modulate blood pressure. Use of endoscopic therapies has been described in a small number of patients. Esophageal varices may be amenable to band ligation. Argon plasma coagulation (APC) and radio-frequency ablation (RFA) have been associated with a partial response in a few patients; however, extensive GI tract involvement, including sites beyond the reach of an endoscope, may limit the broad application of these techniques. Finally, bevacizumab, an intravenous medication indicated for treatment of some forms of cancer, appeared to have a dramatic effect on GI bleeding in one of two individuals who were treated with it in a recent report [9]. More data are required to help clarify the role of this agent as a therapy for bleeding from GI telangiectasia.

Pulmonary Vascular Malformations

Hepatopulmonary syndrome is described as pulmonary vascular dilation due to liver disease of any form that leads to a deficit in arterial oxygenation [15]. It can occur in patients with or without portal hypertension. Individuals with DC are at risk of hepatopulmonary syndrome due to the increased frequency of underlying liver disease in this population [14]. However, pulmonary arteriovenous malformations (PAVMs) in the context of DC have been reported with and without underlying hepatopulmonary

syndrome [1, 16-18]. In DC, PAVMs may be microscopic and multiple, making diagnosis and treatment challenging [1, 16]. Macroscopic pulmonary AVMs have been noted in at least one patient with DC [1]. In general, PAVMs lead to right-to-left shunting of blood, which causes a deficit in arterial oxygenation and progressive respiratory insufficiency if undetected and untreated. Both of these manifestations can present with non-specific symptoms such as dyspnea on exertion, clubbing of the digits, cyanosis, or abnormal pulmonary function tests (PFT). The diagnosis of PAVMs may be missed or delayed as it may present with symptoms similar to those of pulmonary fibrosis, a well described complication of DC (see Chapter 14, Pulmonary Fibrosis) [19]. However, unexplained clubbing and lung diffusion capacity (DLCO) abnormality out of proportion to the degree of pulmonary fibrosis must alert clinicians to the possibility of PAVMs or hepatopulmonary syndrome (in the presence of liver disease).

Bubble echocardiography is a diagnostic modality that detects presence of right-to-left shunting and pulmonary vascular malformations. Further invasive testing such as angiography or cardiac catheterization may be necessary to confirm the presence of these abnormalities, along with a workup to rule out cardiac causes of these symptoms.

Management

At present, there are no specific recommendations for treatment of DC-associated vascular malformations. Testing for other genetic syndromes, such as hereditary hemorrhagic telangiectasia, should be considered in the differential diagnosis for multi-organ vascular telangiectasias. Management guidelines follow medical and surgical recommendations appropriate for each entity, for example, photocoagulation for retinal telangiectasias, and coiling or nifedipine for PAVMs. Currently, the only known successful treatment for hepatopulmonary syndrome is liver transplantation (Chapter 18, Hepatic Complications and Chapter 19, Liver Transplantation) [15].

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Chapter 17

Gastrointestinal Disease

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Introduction

Telomere biology disorders (TBD) affect rapidly dividing tissues including the skin and bone marrow. The luminal gastrointestinal (GI) tract refers to the tubular structures from one's mouth to the rectum (e.g. esophagus, stomach, and intestines). The epithelium (lining) of these structures is another high turnover compartment and may also be a site of disease in TBD.

The penetrance of GI disease in TBD is incomplete, and its prevalence varies. In a cohort of predominantly children, GI disease was estimated to affect approximately 16% of individuals. There are three well-described GI luminal manifestations of telomere-mediated disease: esophageal stenosis, enteropathy primarily affecting the small bowel, and enterocolitis which primarily affects the colon [1]. The latter predominantly affects infants and young children. Gastrointestinal bleeding related to vascular lesions in the GI tract are discussed separately in Chapter 16, Vascular Complications.

Esophageal Stenosis

Presentation

Esophageal stenosis is a narrowing of the esophagus that may interfere with swallowing. It is one of several examples of luminal stenotic lesions that appear in classic dyskeratosis congenita (DC). Lacrimal duct and urethral stenosis may also occur (see Chapter 7, Ophthalmic Manifestations and Chapter 20, Genitourinary Complications). The prevalence of esophageal stenosis in DC is not known, but many of the reported patients are children with classic mucocutaneous features. In cases where the esophageal stenosis is severe and congenital, esophageal stenosis may manifest soon after birth as poor feeding, regurgitation and failure to thrive. In older children and adults, individuals may develop adaptive mechanisms such as thorough chewing and selective food avoidance. This may be because the narrowing develops over time. Regardless, a high index of suspicion in young children and an explicit and detailed swallowing history in older children and adults is often necessary to elicit the symptoms in chronic cases. In addition to stenoses, esophageal webs (a thin membrane that grows inside the esophagus) and Schatzki rings (a circular band of mucosal tissue that can form at the end of the food pipe closest to the stomach) have been described in DC and other TBD.

Diagnostic Workup and Treatment

The ideal initial evaluation for esophageal stenosis is a cine esophagram (video contrast swallow study). It is typically done under the supervision of a speech therapist. This study is preferred to a static barium swallow evaluation which may miss subtle swallowing difficulties because it is not supervised by a speech therapist. Interpretation of these diagnostic studies should include a thorough and focused evaluation of the cricopharynx and proximal esophagus as these regions are frequent sites of stenosis in DC.

Once the location of the stenosis is identified, endoscopic evaluation is necessary to confirm the diagnosis and proceed with therapeutic dilatation. Other causes of obstruction may also be ruled out at this time, including head and neck squamous cell cancers. In cases where the obstruction is proximal, it may be important to have input from an interventional gastroenterologist specializing in the esophagus (i.e. esophagologist) or an otolaryngologist (ears, nose, & throat specialist). Stenoses may at times be severe and, in these cases, pediatric endoscopy equipment may be required for dilatation in symptomatic adults.

Once completed, esophageal dilatation can significantly relieve symptoms. Multiple dilatations may however be required if symptoms recur and have been performed successfully in several cases.

Enteropathy

Presentation

Enteropathies often present with subtle and chronic complaints. Symptoms may include nausea, early satiety, non-specific abdominal pain, food intolerance, difficulty with weight gain, diarrhea, and food allergies. In extreme cases, it may present with failure to thrive. TBD-associated enteropathy can cause significant morbidity even though it is, in most cases, not life-threatening. Its precise prevalence is unknown since the symptoms often overlap with symptoms of irritable bowel syndrome and as outlined below the pathologic findings are patchy and may be missed by localized biopsies.

Diagnostic Evaluation and Treatment

In cases where the onset of symptoms is relatively recent, a diagnostic work-up that excludes other pathology, such as infection or malignancy, should be performed. This may include laboratory evaluation, upper endoscopy with biopsies of the proximal small bowel, and colonoscopy with biopsies, even in the absence of gross pathology. A

specialized pathologist may need to review these specimens to evaluate for subtle findings. The histopathology may reveal intraepithelial lymphocytosis, villous atrophy, and increased apoptosis. These findings are non-specific and are also seen in celiac disease among other enteropathies.

In some cases, affected patients adjust their diet spontaneously in response to their symptoms, thus self-treating their symptoms. There is anecdotal clinical experience that a gluten-free diet may improve symptoms even in patients who do not fulfill typical diagnostic criteria for celiac disease. In severe cases, weight loss and malabsorption may occur and require aggressive nutritional support. Parenteral (intravenous) nutrition has been prescribed with variable degrees of success in achieving nutritional rehabilitation.

Individuals with TBDs may develop enteropathy after solid organ or hematopoietic cell transplantation. This may be related to transplant preparative regimens, immunosuppressive medications, or graft-versus-host disease. In cases where the enteropathy is exacerbated by medications (e.g., mycophenolate mofetil), discontinuing the offending agent may be necessary [2]. A multi-disciplinary evaluation and familiarity with the telomere-associated histopathology is ideal to formulate a treatment plan.

Enterocolitis

Presentation

Enterocolitis is a serious and life-threatening GI complication of TBDs and is generally limited to infants and young children. It is particularly prevalent in Hoyeraal-Hreidarsson (HH) syndrome and may be one of its initial presentations and defining features. Enterocolitis is marked by abdominal pain, failure to thrive, and bloody diarrhea. In some cases, bacteremia, sepsis, and bowel perforation may occur. The features of TBD-related enterocolitis overlap with those of inflammatory bowel disease (IBD), especially ulcerative colitis. In fact, some of the same genes associated with TBD have

also been implicated in development of very early onset IBD [3], a rare subset of IBD which has monogenic underpinnings, unlike the complex genetic pattern observed in most individuals with IBD. The pathophysiology of this condition likely reflects epithelial-intrinsic defects as well as severe immune system abnormalities including those involving B cells.

Diagnostic Workup and Treatment

The diagnosis of enterocolitis is a clinical one and based on the patient's age and symptoms. Colonoscopy often reveals friable mucosa, gland drop-out, and inflammation. Treatment is supportive including bowel rest, antibiotics, and nutritional support. Often parenteral nutrition is prescribed. In cases of bowel perforation, surgical intervention is required. It is unclear whether immunosuppressive therapies that are used for IBD are helpful in these settings and there may be potential risks of giving immunosuppressive medications (e.g., TNF-alpha inhibitors) to patients with HH since they have an underlying intrinsic immune disorder. Immune reconstitution with hematopoietic cell transplantation has been performed in children with HH or DC who have enterocolitis, but it is unclear from the authors' experience to date, whether it is possible to completely reverse this GI complication. Overall, this condition, when it is severe, may be associated with poor prognosis.

Acknowledgements

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Chapter 18

Hepatic Complications

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Introduction

Hepatic involvement in dyskeratosis congenita (DC) and related Telomere Biology Disorders (TBDs) may vary from mildly abnormal liver tests to advanced cirrhosis, portal hypertension and hepatocellular carcinoma. The time of onset of liver involvement also varies and depends on the mutated gene, the type of pathogenic variant, length of telomeres, genetic anticipation, and interaction with environmental factors.

Overview

As indicated in Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders, many patients with hepatic involvement are older and carry mutations in the *TERT* or *TERC* genes [1-3]. In fact, in patients with *TERT* or *TERC* mutations, who may not have the usual skin, mucosal and nail abnormalities that characterize DC, liver disease may be the only clinical manifestation of a TBD [4]. In other cases, liver disease can accompany aplastic anemia or be found in relatives of patients with aplastic anemia who are otherwise silent carriers of a TBD.

However, individuals with DC/TBD are at high risk of developing liver disease in their youth. Children with DC who undergo hematopoietic cell transplantation (HCT) for marrow failure in the first decade of life may be particularly prone to the HCT complication of veno-occlusive disease, and should be monitored carefully for cirrhosis after transplant [5]. Modern reduced intensity conditioning regimens may reduce the risk of liver toxicity [6].

Additionally, cryptogenic cirrhosis is found in a small proportion of patients with idiopathic pulmonary fibrosis, implicating telomere erosion in both fibrotic processes [7]. It is important to note that the same telomerase mutation may manifest differently in different individuals of the same family; whereas some may develop liver disease, others may be diagnosed with aplastic anemia or idiopathic pulmonary fibrosis [8].

The pattern of hepatic involvement is variable. The most common liver pathologies associated with telomere disorders are described below.

Cirrhosis

Cirrhosis is a late stage of progressive liver fibrosis and is characterized histologically by distortion of hepatic architecture and formation of regenerative nodules [9]. Although historically histology had been used to confirm the diagnosis of cirrhosis, liver biopsy

has now been successfully replaced by noninvasive fibrosis assessment tools, such as transient elastography or magnetic resonance elastography, in patients with early compensated disease.

In early stages, individuals are often asymptomatic and the diagnosis of cirrhosis may be suspected on the basis of abnormal liver chemistries or imaging.

In late presentation, individuals may complain of chronic fatigue, jaundice (yellowing of the eyes and skin), hematemesis (vomiting blood), ascites (fluid distension in the abdomen), peripheral edema (swelling of legs and feet), and in more advanced cases, symptoms of hepatic encephalopathy, including sleep-wake cycle reversal, disorientation, confusion, and even coma. Physical examination may reveal signs of hepatic insufficiency, such as jaundice, spider telangieactasias (small dilated blood vessels visible in the chest), palmar erythema (redness of the palms), gynecomastia (breast enlargement), and/or signs of portal hypertension, including splenomegaly (enlarged spleen), ascites, or asterixis (flapping tremor). Laboratory tests frequently show elevated hepatocellular enzymes and alkaline phosphatase.

At late stages, synthetic liver function may become impaired, resulting in low serum albumin (largest circulating protein in the blood), and prolonged prothrombin time (reflection of decreased hepatic production of clotting proteins).

Finally, imaging of the liver reveals a nodular hepatic surface with increased echogenicity as well as signs of portal hypertension, including splenomegaly and portosystemic collaterals, such as gastroesophageal varices.

Although the pathogenesis of cirrhosis is not completely understood, it appears that telomere attrition plays an important role. Chronic liver injury stimulates hepatocellular proliferation, cell turnover and progressive telomere loss, which in turn promotes cell proliferation arrest and apoptosis [10]. In fact, telomere shortening by itself is associated with cirrhosis formation [11].

Therefore, cirrhosis is not only a direct consequence of TBD, but *TERT* mutations also are risk factors for cirrhosis development in patients with chronic hepatitis C infection or alcohol-associated liver disease [10]. *TERT* mutations are more prevalent in patients with these conditions than in the normal population. However, it is not clear whether disease may be more severe when a *TERT* mutation is present.

Non-Cirrhotic Portal Hypertension

Although cirrhosis is the most common cause of portal hypertension, approximately 10-15% of individuals with clinically significant portal hypertension do not have advanced liver fibrosis. A variety of morphologic changes in the liver tissue may result in an increase in portal pressure, such as nodular regenerative hyperplasia (NRH) [12].

The association of NRH and TBD-related mutations has been described in several families, with or without the presence of bone marrow failure and/or pulmonary fibrosis [13, 14].

Like cirrhosis, patients with NRH are often asymptomatic in early stages of disease, but a large proportion go on to develop complications of portal hypertension, including ascites and gastroesophageal varices. As opposed to cirrhosis, however, synthetic liver function is typically preserved in NRH due to absence of fibrosis.

Pathology

When liver biopsy is performed, histology may reveal distortion of the hepatic architecture, with bridging fibrosis (fibrosis connecting portal areas) or perisinusoidal fibrosis. Inflammatory infiltrate is another common feature, and macrovesicular steatosis and Mallory bodies may be noted. Additionally, sinusoidal endothelial cells around the portal areas and central veins may stain positive for CD34, which suggests abnormal arterial blood flow to the sinuses. Mild iron accumulation in hepatocytes is also usually noted. On the other hand, nodular regenerative hyperplasia (NRH) is

histologically characterized by small regenerative hepatic nodules in the absence of significant fibrosis [15]. CD34 may be positive in sinusoidal endothelial cells, which is consistent with portal hypertension.

Hepatopulmonary Syndrome

Although pulmonary fibrosis and emphysema are the most common respiratory complications of TBD, patients with portal hypertension (cirrhosis- or NRH-related) are at increased risk for hepatopulmonary syndrome (HPS). HPS is a vascular complication of liver disease characterized by low pulmonary vascular resistance secondary to intrapulmonary vasodilatation and shunting [16]. The prevalence of HPS in noncirrhotic portal hypertension is estimated to be around 10% [17], and patients with TBD may be at a higher risk [18]. HPS is manifested by progressive shortness of breath, worse in upright position, and hypoxemia (low oxygen levels in the blood). No directed therapy for HPS currently exists, and treatment is limited to liver transplantation with an expectation of resolution of hypoxia in patients post-transplant.

Hepatocellular Carcinoma

Development of hepatocellular carcinoma in patients with telomerase mutations has been reported [10, 19]. However, the number of cases reported so far is too small to determine whether the clinical behavior or tumor aggressiveness differs in patients with TBD compared to the general population. It appears that the pattern of liver damage is similar to the involvement seen in hematopoietic tissue, in which telomere dysfunction results in organ failure and subsequent malignant transformation.

Other Manifestations

Hepatic veno-occlusive disease may be a complication following HCT for aplastic anemia in patients with DC [5]. Some patients with telomerase mutations have also

been found to have hepatic steatosis (fatty liver) in the absence of risk factors, such as excessive alcohol use or metabolic syndrome [3].

Monitoring Liver Involvement in TBD

Individuals with TBD, including dyskeratosis congenita, should be screened for liver involvement at diagnosis and monitored approximately once a year, depending on the patient's specific clinical manifestations. Complete liver chemistries (aminotransferases, alkaline phosphatase and total bilirubin), as well as markers of synthetic liver function (prothrombin time and albumin) should be performed.

Abnormal liver tests or physical examination findings suggestive of advanced liver disease (as outlined above) should prompt for additional testing, including abdominal ultrasound and/or noninvasive fibrosis assessment (with liver and spleen stiffness measurement). Similarly, MR elastography or transient elastography should be obtained in the presence of additional risk factors for advanced liver fibrosis, including metabolic syndrome, alcohol misuse and/or chronic hepatitis C. Transjugular liver biopsy with hepatic venous pressure gradient measurement may be needed if other tests are inconclusive.

The liver is also of major concern for side effects of polypharmacy. Patients should always inform their medical team of all prescribed medications. Patients taking androgens are particularly susceptible to developing liver complications, although more recent work has not found an increased rate of abnormal liver tests in these patients (see also Chapter 10, Medical Management of Bone Marrow Failure in Telomere Biology Disorders) [20].

Surveillance of hepatocellular carcinoma, in the form of abdominal imaging (ultrasound, CT or MRI) should be obtained every 6 months in those with established cirrhosis. Screening for hepatopulmonary syndrome is not indicated, except in the presence of respiratory symptoms or complaints. The diagnosis of HPS should be suspected in the

presence of hypoxia or hypoxemia with elevated alveolar-arterial gradient. Contrast-enhanced echocardiogram should be used to confirm the presence of intrapulmonary right-to-left shunting.

As in other complex multi-organ diseases, several subspecialties should be involved in the care of patients of TBD, and referral to an expert hepatologist is recommended for all individuals with TBD.

Treatment Options

There is no specific treatment for liver disease in TBD. Although androgens can be used to improve cytopenias in patients with TBD, studies remain inconclusive about their liver-specific benefits [21], and patients receiving androgens should be carefully monitored for liver toxicity. In more severe cases of liver disease or the development of hepatocellular carcinoma, liver transplant may be an option, which is increasingly described in the literature [3, 19, 22-26].

Cirrhosis and portal hypertension are managed as they are for other etiologies, with a focus on prevention and treatment of additional liver and other organ injury, and management of symptoms. Hepatic veno-occlusive disease following HCT is also managed in patients with TBD as it is in patients undergoing HCT for other indications. Progression of liver disease may lead to gastroesophageal varices and bleeding, portal hypertensive gastropathy, ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, hepatocellular carcinoma, and/or hepatopulmonary syndrome.

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Chapter 19

Liver Transplantation

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Introduction

Liver transplantation (LT) is used successfully both in children and adults with acute liver failure or in patients with complications of decompensated chronic liver disease in the hopes to extend survival with excellent quality of life [1, 2]. In the past decade, liver transplant has also been reported in a small number of patients with dyskeratosis congenita (DC)- and Telomere Biology Disorder (TBD)-associated severe liver disease. Although these reports are few, they do highlight the potential benefit of liver transplant in selected patients with DC/TBD and related hepatic complications [3-9].

Liver Transplant History

The first liver transplant was performed in a child in the 1960s by Dr. Thomas Starzl at the University of Colorado [10, 11]. Since then, substantial scientific progress in liver transplantation involving surgical techniques, postoperative management and immunosuppression treatments have significantly improved transplant patient and graft survival. Over 8000 liver transplants are performed yearly in the United States alone with survival rates at 5 years post-transplant ranging from 70-90% in children and adults [1, 2, 10-12].

Indications for Liver Transplant

There are several reasons that a patient may be referred for liver transplant consideration [1, 2, 10, 11]. In some patients, LT may be indicated due to primary, non-metastatic liver tumors or liver-centered metabolic diseases which are ineligible or refractory to medical or surgical intervention. In others, LT is considered when there is acute decompensation or chronic liver failure with significant liver dysfunction evidenced by abnormal serum albumin, bilirubin and INR/PT, or hyperammonemia, leading to hepatic encephalopathy. LT may also be indicated secondary to complications of chronic liver disease such as intractable ascites, variceal bleeding, and other liver-associated complications of portal hypertension like hepatopulmonary or portopulmonary hypertension. Additionally, pediatric patients may also be referred for LT if chronic liver disease causes poor weight gain/ growth failure. Timing for LT referral depends significantly on the clinical situation. Referral for may be emergent, urgent, or anticipatory.

DC/TBD and Liver Transplant

LT has been thus far reported in a small number of pediatric and adult patients with DC/TBD (Table) [3-9]. Progressive dyspnea and hypoxia suggestive of hepatopulmonary

syndrome were present in all patients and represented one of the main indications to LT. Decompensated cirrhosis with ascites and varices was also present in 3 of the 5 described patients. In these reports, the length of longitudinal follow-up periods varied from a few months to up to 10 years in one case. At the time the cases were published, all patients were reportedly alive with resolution of the complications of their DC/TBD-associated liver disease.

Liver Transplant Evaluation

In general, a formal liver transplant evaluation at an experienced transplant center aims to determine if a liver transplant will be useful at that time, exclude potential contraindications to transplant, and educate the patient and caregivers about the transplantation process, benefits, and risks [1, 2]. In order to accomplish these goals, a LT evaluation involves the following:

- Confirmation of diagnosis and extent/severity of the liver disease and its complications.
- Determination of the relative urgency of the liver transplantation.
- Identification and assessment of systemic comorbidities and suggestion/coordination of management plans to optimize patient's status prior to potential liver transplantation.

A liver transplant evaluation consists of a large multidisciplinary team who uses their expertise to tailor the liver transplant evaluation/investigation to the specific needs of the patient. This team typically involves many specialists including a transplant surgeon, transplant hepatologist, transplant coordinator, infectious disease specialist, social worker, dietician, transplant pharmacist, transplant anesthesiologist, psychologist/psychiatrist, and transplant financial coordinator. Depending on the patient's clinical situation, the LT evaluation may also require consultation with additional specialists such as a cardiologist, pulmonologist, nephrologist, hematologist, genetic/metabolic specialist, dentist, etc.

To confirm the current extent and severity of the primary disease and the patient's multiple organ systems, the LT evaluation will involve laboratory and diagnostic studies and review of medical, surgical and pathology reports. If additional assessment is required through additional testing or new subspecialty consultation, this will be ordered as part of the transplant evaluation. This extensive evaluation will allow the liver transplant team to have a clear assessment of the patient's liver, cardiopulmonary, renal, immunological, and nutritional status.

The psychosocial assessment is another fundamental aspect of the transplant evaluation, as lifelong care is a prerogative of successful liver transplant. In this sense, both psychological and logistical barriers to medical adherence need to be identified and addressed prior to transplant to avoid a negative impact on outcomes. In adult patients, a continued destructive behavior resulting from drug and alcohol addiction may represent a contraindication to transplantation. For these reasons, psychologists, social workers, and psychiatrists are part of the liver transplant team, ensuring that social and psychological supports systems need to be in place for patients and their family.

In the LT evaluation process, it is also critically important to identify any contraindications for transplant, conditions such as extrahepatic malignancy or systemic infection, which make the patient high risk for transplant at that time, likely to develop potential complications after LT, or where a patient's overall condition is thought unlikely to benefit from a LT.

At the end of the liver transplant evaluation, each transplant center's multidisciplinary team comes to a consensus decision regarding the indication, severity and urgency for liver transplant. Each transplant center makes a team determination if the patient is likely to benefit from LT at that time, and, if so, will list the patient for transplant if the patient/family agrees. This determination of transplant listing eligibility is center-dependent and may differ between transplant centers. Patients and their families

may repeat the entire transplant evaluation process at different transplant centers, with different determinations of eligibility.

For patients with DC/TBD and pulmonary symptoms, cardiopulmonary assessment through cardiology and pulmonary consultation and specific imaging (e.g., computer tomography, bubble contrast echocardiography and/or albumin lung perfusion scan) is particularly important to evaluate the degree of pulmonary complications like pulmonary fibrosis, arteriovenous malformations, and hepatopulmonary syndrome (HPS). The distinction between these entities is fundamental as HPS may be reversible with liver transplant while pulmonary fibrosis is not and can impact a patients' eligibility for liver transplant as well as complicate the post-transplant course. LT is appropriate for the treatment of HPS in children with cirrhotic liver disease. For patients with noncirrhotic liver diseases, as in some patients with DC/TBD, consideration of alternate non-transplant therapies, such as occlusion of portosystemic shunt by surgical or interventional radiology approaches, should be explored. LT may be indicated in those patients who are not eligible for these interventions.

Types of Liver Transplant

Transplant livers may come from a deceased donor (whole liver or partial segment of a liver), or, a living donor who donates a segment of their liver (Figure) [1, 2, 10-12]. Given that organ scarcity remains the major limiting factor in liver transplantation, the advent of technical innovations have made it possible to safely transplant only segments of liver from deceased and living donors. This has further expanded the pool of available organs, significantly reducing the wait list mortality in children.

- **Whole liver:** A full liver is transplanted from a size matched deceased donor. Donor-to-recipient size mismatch is a limitation to this type of transplant.

- **Reduced size graft:** A whole liver is reduced in its size to match the recipient.

- **Split liver graft:** A whole liver is naturally divided into two sections. Depending on the size of the recipient, a section of the liver can be obtained from an adult deceased or living donor. In infant/toddler pediatric patients, a portion of the left lobe (the left lateral segment) of a deceased or living donor may be considered for transplantation.

- **Living-donor liver transplant (LDLT):** Either the left/ left lateral segment or right lobe of the liver can be used for transplantation, depending upon anatomic considerations and the size of both the donor and recipient liver. Both donor and recipient livers grow and regenerate within weeks to months.

Studies comparing these different types of LT have found a higher rate of perioperative complications with this last technique [1]. Long-term patient survival, however, seems comparable with that of deceased whole liver transplantation [1, 10]. If a patient is considered appropriate for listing for LT, the appropriateness of whether they can receive a living donor depends on the existing anatomy of the recipient patient, the organ size/associated tissues the patient requires, and the surgical risk of a living donor transplant to the recipient and donor. For LDLT to be appropriate, three things must be strongly considered:

1. The likelihood of the recipient's long-term survival must be high.
2. The risk of mortality to the donor must be low.
3. The donor must be well informed of all the potential risks of undergoing the donation and still agree to undergo the surgery of their own free will.

Thus, LDLT is typically considered when deceased donor LT is not an option or a deceased donor LT organ has not become available. The determination if living donor LT is appropriate for a patient is transplant center-dependent and can differ between centers. In addition, living-donor transplantation is not performed at every LT center, and its availability should be discussed at the time of initial evaluation.
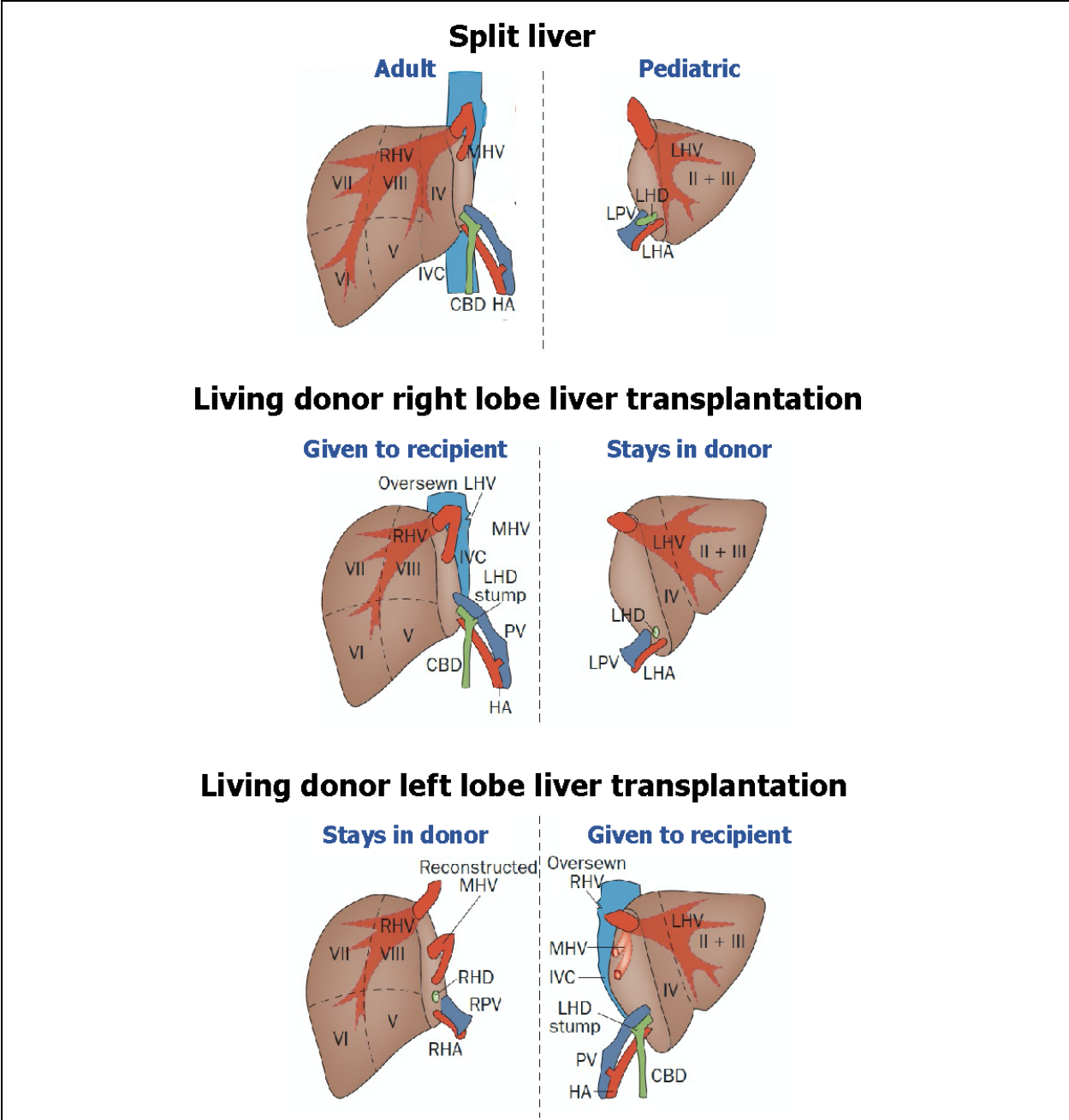
Figure 1. Types of Liver Graft.
IVC: inferior vena cava; HA: hepatic artery; PV: portal vein; CBD: common bile duct; LHA: left hepatic artery; LPV: left portal vein; LHD: left hepatic duct; LHV: left hepatic vein; RHA: right hepatic artery; RPV: right portal vein; RHD: right hepatic duct; RHV: right hepatic vein; MHV: middle hepatic vein.

* Figure modified from Zarrinpar A and Busuttil RW, *Nat Rev Gastroenterol Hepatol*. 2013 [12].

Deceased and Living Donor Selection

When an organ from a deceased donor becomes available, the transplant team carefully reviews the donor's clinical and biochemical characteristics to assess the suitability of the transplant with regards to the specific potential recipient [1, 2, 10, 11, 14]. The donor's blood type, age, infectious status, intensive care hospitalization time, hemodynamic stability, and estimate of liver fatty infiltration are some of the many important factors which are considered, as they have been found to significantly impact transplant outcomes. In addition, particular attention is also paid to the size of the donor liver given that an adequate parenchymal volume is fundamental for the success of the transplant.

When a living donor is considered, the primary focus of the donor evaluation is the donor's safety. For this reason, the team evaluating the donor should be different from the recipient's team in order to avoid bias and conflicts of interest. Living-donor LT programs may require donors to be healthy adults, typically between 18 to 60 years of age with compatible blood type, normal liver tests, and appropriate medical and surgical past medical histories. Exact donor acceptance criteria may vary slightly between centers. If these criteria are met, then the potential donor may meet with the transplant surgeon and transplant hepatologist to discuss the surgical and medical details of LDLT. If the potential donor voluntarily expresses continued interest in being considered for live-donor transplantation, the donor candidate will then be referred for a complete medical and psychosocial assessment. This will entail several clinic visits, additional blood work, and abdominal imaging, which allows better characterization of the donor liver anatomy, and procedures like a liver biopsy. Additional testing to assess the potential donor's multiple organ systems may also be required to assess the donor's safety to undergo the operation. The typical evaluation process usually takes between 2 and 4 weeks. Once all the information has been gathered, the donor's evaluation team determines the donor's safety and suitability to undergo a LDLT from a medical, surgical, and psychological standpoint.

Although parents and siblings of patients can volunteer to be candidates for a LDLT, if they are carriers of a genetic variant for the recipient's liver condition, such as potentially in families of patients with DC/TBD, other donor options may be necessary due to the concern for disease recurrence.

Deceased Donor Organ Allocation in the USA

Since 2002, deceased donor liver allocation in the US requires centers to calculate scores which measure the patient's illness severity and the risk of death within three months on the liver waiting list [1, 2]. The Model for End-Stage Liver Disease (MELD) is used to prioritize patients 12 years of age and older for organ allocation in the United States. This formula includes the total serum bilirubin, creatinine, INR, and serum sodium. For patients younger than 12 years, the Pediatric End Stage Liver Disease (PELD) score is used instead, which utilizes lab values (total serum bilirubin, INR, and albumin) and presence of growth failure (height and weight) and an indicator of whether the patient is less than one year of age. A higher score indicates a worse severity of illness and higher risk of death.

Exception points can be requested when the MELD and PELD score are thought to not accurately reflect the patient's condition. Request for exceptions points is submitted to the national review board with supporting clinical documentation. The anonymous board reviews the documentation and decides if the request for exception points will be granted. If granted, the patient is listed using the exception point score. For patients with DC/TBD and indications for transplant like hepatocellular carcinoma and HPS in which the patient's liver function is not significantly impaired, exception points can be particularly useful.

Liver Transplantation Timeline

The preoperative management while awaiting LT is crucial to optimize the patient's clinical condition [1, 2]. Patients can require admission in the hospital during this time or can be managed at home however with close multidisciplinary follow up. While listed, the patient will continue to have outpatient follow-up appointments with the primary hepatology team and the transplant team and continue to get required lab testing and physical exams to renew transplant listing information.

If a suitable deceased-donor organ becomes available or the patient has been scheduled for living-donor liver transplant, the patient is admitted to the hospital prior to the operation. Depending on the recipient's needs during the transplant, the LT operation can take up to 12 hours, after which the patients will be transferred to the intensive care unit (ICU) for close post-operative surveillance and management. Generally, after a few days, most patients can be transferred to a general hospital unit. The duration of the transplant hospitalization may vary considerably depending on the degree of systemic medical conditions of the recipient.

Complications of Liver Transplantation

Complications can occur both early in the postoperative period as well as months and years after a transplant [15-19]. Important transplant complications are briefly reviewed below:

- **Graft primary non function:** This is the most common reason for early retransplantation (a second or subsequent LT). Graft primary non function is characterized by early graft failure which can occur intraoperatively or in the immediate postoperative hours. It is thought to be multifactorial with several factors playing a role such as donor advanced age, hemodynamic instability, sub-optimal donors, cold ischemia time, and reperfusion damage.

- **Vascular complications:** Hepatic artery thrombosis (HAT) is the most common vascular complication and affects pediatric LT recipients 3-4 times more frequently than the adult LT recipients. When HAT occurs early in the post-transplant period, it can lead to ischemic graft damage and may require re-transplantation. Later complications of HAT can lead to biliary ductal complications such as intrahepatic biliomas and biliary strictures. Less commonly, portal vein thrombosis can occur. This is an acute presentation in which patients may show signs of graft failure while later occurring portal vein thrombosis may manifest as signs of portal hypertension with decreased platelets count, splenomegaly, or gastrointestinal bleeding.

- **Biliary complications** remain a common source of morbidity for LT recipients with an estimated incidence of 10-15% in deceased donor transplants and as high as 15-30% in adults and pediatric LDLT or split-liver transplant recipients. Bile duct complications can also include bile leaks, which tend to occur in the early postoperative period, and biliary strictures, which are more common and occur in later stages of transplant. As mentioned above, HAT is one of the main risk factors for bile duct complications post-transplant.

- **Hemorrhage:** When present, it typically manifests within the first 48 hours post-transplantation. It is most commonly treated conservatively but in 10-15% of adult and pediatric recipients might require a return to the operating room for surgical exploration to determine the source of bleeding.

- **Rejection:** If immunosuppressive medications are not given, the recipient's native immune system inevitably recognizes the transplanted liver as a foreign body, triggering an immune response aimed at destroying the graft itself. The process of the recipient's native immune system causing inflammation and injury to the transplanted organ is called rejection. In order to minimize the likelihood of this process, immunosuppressive medications are given with the transplant operation and maintained in the post-transplant period. Determining a patient's adequate

immunosuppression is a continual balance between the risk of rejection and the risk of infection from over-immunosuppression.

Rejection can be:

- o **Hyperacute,** occurring within minutes to hours from the LT. It is usually antibody and complement mediated and generally irreversible.

- o **Acute**, occurring within weeks to months after transplant, but it can also happen at any time after transplant. It is T-cell lymphocyte mediated and generally responds to currently available immune suppressants. Patients present with elevated liver enzymes without symptoms, or it is sometimes associated with non-specific symptoms like general malaise or abdominal discomfort. A liver biopsy confirms the diagnosis of acute cellular rejection. The treatment involves measures to increase the patient's level of immunosuppression, which usually involves a short course of high dose IV steroids and increase in baseline immunosuppression level. Switching immune suppressive agent or adding a second drug may also be considered.

- o **Chronic**. It is also T-cell mediated but occurs over months to years after LT. This process involves long-term graft dysfunction and liver fibrosis and manifests as progressive cholestasis and most often does not respond to immunosuppressive medications, causing late graft loss.

- **Infections**: Because of the continuous immunosuppression, which weakens the immune system, LT recipients are at risk for opportunistic infections including viruses (especially cytomegalovirus, Epstein-Barr virus, and herpes zoster and simplex), bacteria (such as *mycobacteria*, *listeria*, and *Nocardia*), and fungi (including *Pneumocystis jirovecii*, *Aspergillus*, and *Cryptococcus*). Moreover, prolonged hospitalization and invasive procedures may lead to nosocomial (hospital acquired) bacterial infection, such as pneumonia, cholangitis,

bacteremia, or urinary tract infection. Strategies to limit the infectious risk of these patients include prophylactic use of antimicrobials in selected cases, optimization of immunization status when possible, and avoidance of high-risk exposures. Transplant teams constantly strive to find a careful balance between the risk of underimmunosuppression, and therefore rejection, with the risk of overimmunosuppression, and therefore infections and medications side effects.

- **Post-Transplant Lymphoproliferative Disorder (PTLD):** Typically involves uncontrolled B cell proliferation and includes a heterogeneous group of disorders ranging from benign lymphatic hyperplasia to lymphoma. Studies have shown that the degree of T-cell immunosuppression and recipient EBV serologic status represent the main risk factors of PTLD. Specifically, EBV-negative recipients of EBV-positive donor organs are at highest risk of developing PTLD. It is more common in pediatric patients likely because a higher percentage of children are EBV-seronegative prior to transplantation. PTLD treatment consists of reduction or complete withdrawal of immunosuppressive medications, administration of antiviral drugs, and in the most severe cases, administration of chemotherapy or irradiation, and monoclonal antibody therapy such as rituximab.

Long-Term Management of Liver Transplant Recipients

All LT recipients require lifelong monitoring and management by liver transplant teams in order to ensure (1) graft health, (2) adequate immunosuppressive treatment with minimization of its long-term toxicities and related complications, (3) surveillance of potential recurrence of primary liver disease and, in general, to (4) promote health after LT [15, 16]. Routine monitoring should be comprehensive with scheduled clinic visits and blood work which occurs as frequently as weekly in the immediate post-transplant period and later can be progressively spaced out in stable patients. Imaging and serial histological evaluations are also part of the graft surveillance.

Immunosuppression

Immunosuppression is one of the cardinal determinants of a successful liver transplantation with the goal to prevent rejection and therefore loss of the graft [15, 16]. The management of immunosuppression is tailored based on the patient and their comorbidities, the indication for liver transplant, and the time from transplant. Generally, the priority in the early pre-transplant period is avoiding rejection, while later on limiting long-term immunosuppression side effects and complications becomes more relevant.

In the immediate transplant period, patients usually receive a combination of high dose immune suppressive medications to minimize the body's reaction to the new, foreign liver. Although there is no universally accepted immune suppressive regimen for liver transplant recipients and combinations of different drugs are possible, calcineurin inhibitors are often the cornerstone of long-term immunosuppression maintenance. At the time of transplant, some patients might also receive a so-called induction therapy with basiliximab, a monoclonal antibody against interleukin-2 receptors, which inhibits T-lymphocyte proliferation in addition to steroids and calcineurin inhibitors. Systemic steroids can often be discontinued within weeks to month after transplant. Calcineurin inhibitors are a class of medication which works by inhibiting T-lymphocytes and includes cyclosporine and tacrolimus. Cyclosporine, first used in the 1980s, allowed for tremendous progress in transplant surgery when it was found to dramatically decrease graft rejection. Over the years cyclosporine has been largely replaced by tacrolimus which is more effective in preventing rejection, and in comparison to cyclosporine less likely to be associated with side effects such as gingival hypertrophy, hirsutism, nephrotoxicity, neurotoxicity, and hypertension. Both cyclosporine and tacrolimus are most commonly dosed twice a day and can be taken by mouth as liquid formulation or capsule. A patient's immunosuppression is monitored with serial blood draws to assess the drug trough level and subsequently adjust its dose to maintain the desired trough within a recommended target range. A patient's particular immunosuppression drug target range varies according to co-morbidities, risk of infection and timing from

transplant. In general, the level of desired of immune suppression is highest immediately after transplant and gradually decreases over time. Drug level monitoring occurs more frequently in the immediate post-transplant period and can then be spaced out farther apart when patients are more stable.

Mycophenolate mofetil, azathioprine and sirolimus are additional immunosuppressive drugs that can be used in selected patients and may depend on the following reasons: primary indication for liver transplant, rejection history, severe calcineurin inhibitor toxicity, and need of steroid withdrawal facilitation.

All of these immunosuppressive medications need monitoring for signs of side effects. Side effects are more commonly encountered when higher doses are required soon after transplant but must also be monitored long-term for signs of chronic toxicity. Immunosuppressive medications have been associated with increased risk of renal (kidney) dysfunction, hypertension (high blood pressure), hyperlipidemia (increased lipids), diabetes, obesity, and metabolic syndrome. Patients are regularly screened with physical exams, routine blood pressure measurements, and blood work, including creatinine, glucose, and lipid panel to assess renal function and to evaluate presence of diabetes and cardiovascular disease. Moreover, immunosuppressive medications predispose patients to infectious complications and malignancies, both of which require maintaining a high level of suspicion at all times in transplant recipients. Transplant teams work continually to find the best immunosuppression regimen for each patient's specific needs which carefully balances the risks and benefits of immunosuppression.

In the majority of cases, immune suppression is required lifelong to ensure graft survival. In a select minority of liver transplant recipients, the immune system may develop tolerance to the graft, and immunosuppression may possibly be discontinued many years after transplant.

Conclusion

Liver transplant is an option for a variety of liver disorders and has been done successfully in both children and adults [1, 2, 10, 11]. The decision for undergoing liver transplantation requires an in-depth and comprehensive evaluation by a multidisciplinary liver transplant team. Consideration to the short- and long-term risks of the liver transplant surgery and immunosuppression must be made in relation to the patient's specific condition. Liver disease is reported in about 7% of DC/TBD individuals, and there is no specific curative treatment thus far [3-9]. There have only been a small number of case reports published of patients with DC/TBD and severe liver disease who underwent LT. All of these DC/TBD patients presented with progressive dyspnea and hypoxia concerning for hepatopulmonary syndrome alongside, in some cases, decompensated chronic liver disease. At this time, there is no consensus recommendation as to the role of LT in DC/TBD-associated severe liver disease, given the small number of patients described and the lack of long-term post-transplant data. It can, however, be cautiously noted that this small series of patients suggests acceptable early LT outcomes in selected patients.

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Table. Reported cases of liver transplant in patients with DC/TBD

Patient [reference number]	Gender	Genetic variant	Age at DC/TBD diagnosis	BM involvement	Age at liver disease diagnosis	Liver disease	Age at LT	Type of LT	Additional information	Post LT-follow up
1 [4]	M	TERT p.Lys1050Asn	35 y/o	Normal complete blood counts	N/A	HPS, Splenomegaly, NRH	40 y/o	N/A	Normal lung parenchyma at time of liver disease diagnosis	Hypoxia and dyspnea resolved within 3 months after LT. Developed IPF 12 years after LT.
2 [4]	M	RTEL1 p.Arg1010X	49 y/o	Normocellular BM	N/A	HPS, splenomegaly, intractable ascites, NRH	53 y/o	N/A	Mild lung fibrosis at time of liver disease diagnosis	Hypoxia and dyspnea resolved within 3 months post LT. Within 18 months pt again O2 dependent.
3 [5]	M	Not reported	27 y/o	BMF during teenage years, transfusion dependent, HCT not pursuable given severe liver disease	Approx. 20 y/o	Decompensated cirrhosis, HPS	29 y/o	Whole liver	At time of HPS diagnosis pt was also found to have evidence of fibrotic lung disease and was also considered for lung transplant	Pulmonary function improved significantly with minimal oxygen needs; pt removed from lung transplant list. No further transfusion requirements. Follow up duration N/A.

Patient [reference number]	Gender	Genetic variant	Age at DC/TBD diagnosis	BM involvement	Age at liver disease diagnosis	Liver disease	Age at LT	Type of LT	Additional information	Post LT-follow up
4 [7]	M	Unknown, clinical diagnosis	24 y/o	BMF requiring multiple transfusion. HCT at age 25 y/o	31 y/o	Decompensated cirrhosis, HPS	34 y/o	LDLT (left hemiliver)	Chest CT with ILD involving b/l lower lobes and apices	22 months post LT, no supplemental O2 requirements, normal PFTs. Chest CT with stable ILD.
5 [8]	M	TINF2 c.845G>A	5 y/o	BMF requiring HCT at 28 months of age	5 y/o	HPS, splenomegaly. No histologic finding of cirrhosis.	5 y/o	Combined lung and LT	Rapid worsening of hypoxemia requiring ECMO. No evidence of IPF. Chest CT suggestive of PAVM not suitable for embolization	11 months post LT normal liver function and no hypoxemia

Abbreviations: F:female; M: male; y/o: years old; BM: bone marrow; BMF: bone marrow failure; HCT: hematopoietic cell transplant; LT: liver transplant; HPS: hepatopulmonary syndrome; NRH: nodular regenerative hyperplasia; IPF: idiopathic pulmonary fibrosis; IS: immunosuppression.

Chapter 20

Genitourinary Complications

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Introduction

Several genitourinary complications have been reported in patients with classic dyskeratosis congenita (DC), but there are limited data on their incidence or the extent to which they affect other related telomere biology disorders (TBDs). A review of the United Kingdom based DC registry found that 5% of males with DC had urethral stricture and/or phimosis [1]. In a recent analysis of males with DC at the National Cancer Institute, 10.5% had a history of urethral strictures [2]. While there are a few anecdotal literature reports on kidney abnormalities in DC patients, on review of DC cases registered with the NCI no clear DC associated renal disease was evident [2, 3, 4].

The management of genitourinary complications in individuals with DC should start with a detailed clinical evaluation based on the patient's symptoms and consultation with subspecialists as needed.

Males

Urethral Strictures

Urethral strictures occur most commonly in males. This narrowing of the urethra can occur at any location along the urethra. Men and boys with symptomatic urethral strictures typically present with obstructive voiding symptoms including straining with urination, incomplete bladder emptying, and a narrow or weak urine stream [5]. Patients may have a history of hematuria, frequent urinary tract infections, prostatitis, epididymitis, or bladder stones [6, 7]. The diagnosis of urethral stricture should be made in consultation with a urologist [5, 8]. Imaging studies, such as retrograde urethrography, voiding cystourethrography, or cystoscopy, may be used to determine the location and extent of the stricture.

The pathogenesis of urethral stricture in males with DC/TBDs is not known, but it is hypothesized to be due in part to the limited replicative capacity of the cells in these individuals. In individuals without DC who develop urethral strictures, the normal pseudostratified columnar epithelium of the urethra is replaced by squamous metaplasia. The same mechanism is likely in DC/TBDs, but this has not been studied.

Treatment is determined by the degree of symptoms and location of the narrowing [5, 9, 10] and should be managed by a urologist with experience treating complex urethral strictures. Urethral dilation can be performed for relatively short strictures.

Urethroplasty is used for strictures of the anterior urethra, while meatotomy is the preferred treatment for meatal stenosis.

Phimosis

Phimosis is a relatively common condition in which the foreskin cannot be retracted over the glans penis. The foreskin is not normally retractile in infants but gradually becomes so in childhood [11]. Scarring of the foreskin can result in phimosis, which in turn can cause difficulty in urination, balanitis, or urinary tract infections. Topical corticosteroids may be used for local irritation. Antibiotics may be required for infections.

It is not known if phimosis occurs more often in individuals with DC/TBDs than in the general population. It is thought that it will likely respond to the same management as for individuals without DC, but this has not been systematically investigated.

Hypogonadism

Hypogonadism, manifesting as reduced testosterone production, has been reported in a small number of patients with DC/TBDs. This is further reviewed in Chapter 22, Endocrine and Skeletal Complications.

Females

There are anecdotal reports of labial adhesions, and hymenal and urethral strictures in females with DC. Incidence of these complications in DC/TBDs is not known. However, these should be considered in girls and women with DC/TBDs who have frequent urinary tract infections, difficulty urinating, or abnormal menstrual bleeding. Labial leukoplakia has also been noted in some women with DC/TBDs, but its contribution to adhesions or strictures is not understood. Women and girls with DC suspected of having these complications should be referred to a gynecologist and/or urologist with

experience in treating these conditions. Additional information can be found in Chapter 21, Gynecological and Obstetric Considerations.

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Chapter 21

Gynecologic and Obstetric Considerations

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Introduction

Women with dyskeratosis congenita and telomere biology disorders (DC/TBDs) may benefit from a focus on their gynecologic and reproductive health. In this chapter, we review considerations about routine gynecologic surveillance, special concerns around HCT, gynecologic conditions, fertility, prenatal diagnosis, and pregnancy and obstetric complications. A woman with DC/TBD would benefit from including a gynecologist within her clinical care team to assess her gynecologic and reproductive health.

Overview

A recent publication by Giri et al reporting on the gynecologic issues, fertility and pregnancy outcomes in 39 females with DC/TBDs after menarche suggests that though they undergo menarche and menopause at normal ages as compared to the general population, these women face several key gynecologic problems [1].

First, two thirds of the women present with hematologic symptoms with a significant proportion of those having heavy menses that remains heavy despite using hormonal contraception [1].

Second, some of these women later may undergo hematopoietic cell transplantation (HCT) to treat either bone marrow failure or malignancy. HCT has unique concerns for gynecologic and reproductive health, and may lead to early menopause or infertility for most [2]. Additionally, if a patient undergoes HCT, there is an increased risk for secondary cancers caused by the treatment and an increased risk of lower genital tract HPV disease, but the risk of cervical cancer does not appear to be increased, especially with routine surveillance and posttransplant HPV vaccination [3, 4, 5].

Third, as more women with DC/TBDs become pregnant, information is emerging about their fertility and pregnancy outcomes. Similar to women with other inherited bone marrow failure syndromes, they may have problems with fertility and a higher rate of pregnancy complications related to low blood and platelet counts [1, 6-8].

While nearly all of the women desiring childbearing became pregnant and had a liveborn child, they sometimes had to use fertility assistance to become pregnant and had a higher rate of recurrent pregnancy loss, signs of reduced fecundability [1]. Given the recently reported observations of increased risk of recurrent miscarriage, high rates of preeclampsia, and hematologic complications that can lead to increased preterm birth and primary cesarean section rates, women with DC/TBDs benefit from coordinated care by hematology, gynecology and maternal fetal medicine specialist [1].

Fourth, while women in the general population with telomere shortening are known to have a higher risk of menopause before age 40 or premature ovarian insufficiency and infertility, the recent study of women with DC/TBDs, however, shows natural menopause at a median age of 49 years [1, 9]. Women generally survive into their 50s, underscoring the importance of obtaining necessary screening tests and exams throughout the reproductive years [10]. Though women with DC/TBDs have an increased risk of all types of malignancy, the overall risk of gynecologic cancer does not appear to be increased [3].

Menarche

Women with DC/TBDs attained menarche at normal ages (median 12 years; range 9-17), similar to the general population [1]. Most females begin menarche between the ages of 11 to 16 years, approximately three years after the thelarche, or when the breast buds develop [11]. As can be seen in other bone marrow failure syndromes and chronic disease, young girls may experience pubertal delay due to low body weight, chronic disease, or after HCT. The body requires sufficient body mass and endocrine hormonal signaling to begin menarche. Hormonal signaling can be interrupted by chronic illness and medications, such as oxymetholone (a synthetic anabolic steroid) used in cytopenia. If menses do not occur within 3 years after breast buds develop or by age 16, evaluation by an adolescent medicine specialist or pediatric endocrinologist is warranted [12].

Human Papillomavirus and Cervical Cancer Screening

Human papillomavirus (HPV) is a sexually transmitted virus which can affect squamous cells in the genital area and is associated with genital warts, lower genital tract precancer, and cancer, and oropharyngeal cancer. The currently recommended vaccine licensed by the U.S. Food and Drug Administration (FDA) for prevention of HPV infection is Gardasil9®. The Gardasil9® vaccine prevents nine types of HPV: types 16, 18, 31, 33,

45, 52, and 58 which are associated with 90% of cervical cancer, and types 6 and 11, which cause 90% of genital warts [13]. The vaccine is currently FDA approved for girls/women and boys/men ages 9-45 years [14]. Individuals receive a three-injection series if age 15 years or older, with the subsequent shots given 2 and 6 months after the first, and a two-injection series for girls and boys younger than 15 years, with the second shot given 6-12 months after the first dose [15]. It is not known whether individuals with TBDs or bone marrow failure should receive the three- rather than the two-injection series, but generally those with potentially impaired immunity receive a three-injection series. Adults ages 27 through 45 years who have not been vaccinated may decide to get the HPV vaccine after speaking with their health care provider about their risk of new HPV infections and the possible benefits of vaccination. Vaccination has the greatest benefit if completed prior to the onset of sexual activity.

Abnormal Pap smears and HPV tests are managed by using established guidelines for evaluation and treatment [16]. Patients with an abnormal Pap smear or positive HPV test undergo a procedure called colposcopy, where a clinician, usually a gynecologist, takes a closer look at the cervix and biopsies any areas that appear abnormal. At the time of colposcopy, the vagina and vulva are inspected for other lesions which, if noted, are routinely biopsied as well. Any woman who has biopsy findings of moderate dysplasia or worse warrants treatment. Counseling regarding safe sex practices may help limit exposure to sexually transmitted infections and is important given the impaired immune response in many women with DC/TBDs.

Screening and early detection of lower genital tract squamous cell abnormalities like precancer enables less invasive and successful treatment. The current recommendation for women is to start yearly comprehensive gynecologic exams when they become sexually active or at age 21 [16, 17]. It is reasonable to recommend that women with DC/TBDs have yearly cervical cancer screening especially those with any immune impairment [18]. Additionally, after HCT, annual screening and HPV vaccination is advised [2].

Menstrual Bleeding

Two thirds of the women with DC/TBDs develop hematologic symptoms with a significant proportion of those having heavy menses; despite using hormonal contraception, heavy menses continues in some [1]. Endometriosis is also reported in women with DC/TBDs and is associated with heavy menses. As part of the evaluation of excessive menstrual bleeding in any woman, a complete blood count and pregnancy testing are assessed. An ultrasound may be helpful to exclude other causes of excessive menstrual bleeding including ovarian cysts, or polyps or submucosal fibroids that may form within the uterine cavity.

Mild to moderate menstrual bleeding can usually be controlled with low dose combined oral contraceptives (35 mcg or less of ethinyl estradiol) rather than using high dose estrogen containing pills. These higher dose pills have an increased risk of endometrial atrophy or thinning of the lining of the uterus, which can lead to excessive bleeding with long term use [19, 20]. Heavy menstrual bleeding in the setting of severe thrombocytopenia or bone marrow failure warrants management with hormonal therapy in addition to platelet support. Androgens, commonly oxymetholone or danazol, may be used to treat cytopenia in DC/TBDs (see Chapter 22, Endocrine and Skeletal Disorders).

Patients should have thorough discussions with their care team regarding family planning while on androgens.

Management of Gynecologic Issues During HCT

Approximately 10-30% of DC/TBDs patients undergo HCT for either bone marrow failure or malignancy [1, 3]. Hormonal therapy eliminated symptoms in 97% of patients seen for excessive menstrual bleeding during HCT performed for a variety of indications. Of these women, a single oral contraceptive regimen was effective in 79% [19]. Low dose contraceptives can be given in a transdermal patch, especially in women with poor oral tolerance and elevated liver enzymes [2]. In cases of severe bleeding, high dose oral

contraceptives containing 50 mcg or higher of ethinyl estradiol, or injectable estrogens (intravenous premarin 25 mcg every 6 hours for 24 hours) can be used. These higher doses are maintained until bleeding stops and then treatment is switched to a form of medication that can be continued long-term, such as low dose combined oral contraceptives, or leuprolide acetate [2].

Another class of medications called gonadotropin releasing hormone (GnRH) agonists such as leuprolide acetate, is given by intramuscular injection and has been shown to be effective in suppression of menses in women scheduled for transplant [2]. Injections may, however, be relatively contraindicated in some patients with severe thrombocytopenia because of the risk of bruising or bleeding at the injection site. Patients who experience intolerable hypoestrogenic side effects such as hot flashes or vaginal dryness with leuprolide acetate may benefit from additional treatment with hormone replacement such as low dose combined oral contraceptives.

Patients who have premature ovarian insufficiency, defined as menopause before age 40, that frequently arises from radiation and chemotherapy of HCT may benefit from starting hormone replacement with estrogen and progestins to reach full bone density and maintain sexual function. There are two options in hormone replacement: low dose combined hormonal contraceptives or hormone replacement therapy (HRT), which usually contains lower amounts of hormones than contraceptive preparations. If a woman undergoes premature ovarian failure from HCT before age 35, she may benefit from combined hormonal contraceptives containing at least 30 mcg of ethinyl estradiol to ensure prevention of pregnancy and to maintain bone mass. It has been shown in the general population that women with premature ovarian failure who do not use HRT have increased rates of illness and death compared to women who used HRT [21]. Hormone replacement may help women feel more like their peers and maintain psychological and sexual health.

After HCT, annual cervical cancer screening is advised because of an increased risk of HPV-related disease and to prevent the risk of developing genital tract squamous cell

cancers [22]. Revaccination with HPV vaccine may be considered as an additional strategy to decrease the risk of HPV-related neoplasia [5]. Genital graft-versus-host disease may result in genital scarring in some. Given prior reports of urethral stenosis in DC/TBDs, it is not clear whether the scarring is due, in part, to underlying DC/TBDs (see Chapter 20, Genitourinary Complications) [23, 24]. Other secondary cancers may arise as a result of treatment, because of reactivation of viruses or because the individual has other risk factors for cancers [2, 3, 22].

Fertility and Pregnancy Complications

Recent reports on women with DC/TBDs have expanded the understanding of fertility in this population. Though a small cross-sectional study showed low Anti-Mullerian hormone (AMH) levels in women with DC/TBDs indicative of low ovarian reserve [25], a more recent longitudinal report demonstrates that 25 of 26 women with DC/TBDs who were trying to conceive delivered a live baby illustrating that these women are fertile but experience reduced fecundability [1]. Most of these women in this study who gave birth had a variant in an AD inherited gene other than *TINF2*. Thirteen others in this cohort of 39 women with DC/TBDs had mutations in genes that were either not inherited (*de novo*), AR inheritance, or unknown gene and were less likely to give birth. These women were more likely to have disease-related morbidity that took precedence over family building or were of younger age.

Women with DC/TBDs appear to be at increased risk of pregnancy complications such as recurrent miscarriage, preeclampsia, preterm delivery, increased cesarean delivery rates, maternal transfusion because of low platelet counts, and an increase in postpartum hemorrhage [1]. Because of the increased risks of preeclampsia or worsening bone marrow failure during pregnancy, consultation with a maternal fetal medicine specialist to closely monitor the health of the mother and baby is an important aspect of prenatal care. Serial monitoring of hematologic parameters during pregnancy is warranted to decrease the risk of adverse maternal or fetal outcomes. Based on this

recent study, it appears that bone marrow failure that worsens during pregnancy generally resolves after delivery.

Menopause

In the United States, the average age of menopause within the general population is around 51 years, ranging anywhere from 40-61. Women with DC/TBDs had natural menopause at median age of 49 years (range 46-52) with some undergoing surgical menopause related to endometriosis, uterine prolapse or cervical precancer [1]. Women with telomere shortening as part of ovarian aging appear to be at higher risk of premature ovarian insufficiency [9] though this association has not been consistently reported in women with DC/TBDs. Additionally, women with DC/TBDs who are taking androgens during the transition to menopause may not experience any menopausal signs or be aware that they have experienced menopause.

Options for Family-Building: Cancer Treatment and Fertility

Any woman undergoing cancer treatments or HCT benefits from having discussions with the care team about the potential effect of these treatments on fertility. In some cases, fertility preservation may be possible prior to initiating cancer treatments [2, 26, 27], as reported in the recent study by Giri et al [1]. This process requires evaluation and discussion of the choices with a reproductive endocrinologist or fertility specialist. As part of shared-decision making, there are other family-building options to consider including egg donation, surrogacy, and adoption that are covered in the genetic counseling chapter.

Assisted reproductive technologies (ART), such as in vitro fertilization, to achieve oocyte or embryo cryopreservation independent of HCT, may also be considered by women

with DC/TBDs to enable family building. This technology allows for genetic testing in the embryos. This testing, called pre-implantation genetic diagnosis (PGD), is discussed elsewhere in this book (see Chapter 5, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders). Importantly, ART does not ensure fertility nor will it protect against miscarriage. As TBDs remain lethal diseases in many women, part of shared decision-making regarding fertility preservation may include discussing posthumous disposition of cryopreserved oocytes and embryos.

Malignancy Risk

Individuals with DC/TBD are at increased risk of squamous cell carcinoma [28]. While anogenital cancers in men with DC/TBDs have been reported, to date, only one case of cervical cancer was reported in women with DC [3].

Breast Cancer

There are currently no published reports of increased risk of breast cancer in women with DC/TBDs. Therefore, breast cancer surveillance can conform to the recommendations for otherwise healthy women which includes mammograms and breast exams annually starting at age 40 [29].

Future Research

Although understanding of DC/TBDs has grown tremendously in the past decade, there is still a need for further research to be completed. Main areas of research include further characterization of how telomere biology affects the obstetrical and gynecologic health of women with DC/TBDs and understanding the relationship between DC/TBDs and cancer in women. Further information is also needed on the safety and immunogenicity of HPV vaccination in women with DC/TBDs.

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Chapter 22

Endocrine and Skeletal Disorders

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Introduction

Prospective studies evaluating the endocrine system in individuals with dyskeratosis congenita (DC) and related Telomere Biology Disorders (TBDs) remain limited. Most knowledge regarding endocrine abnormalities is based on reports from the DC/TBDs registry maintained by Dokal et al in the United Kingdom [1] and through clinical observations of the National Cancer Institute's (NCI) prospective DC/TBDs cohort study. Endocrine disorders, such as primary hypothyroidism, growth hormone deficiency (GHD), hypogonadism, or diabetes mellitus, are not common in individuals with DC/TBDs. However, abnormalities related to the skeleton are seen with higher prevalence compared with the general population. Reported abnormal skeletal findings in patients with DC/TBDs include

avascular necrosis (AVN) of the hips and shoulders [2, 3] low bone mineral density [1] and an increased risk of fracture [4]. In addition, therapies that treat hematological manifestations of DC/TBDs, such as androgens and hematopoietic cell transplantation (HCT), may themselves lead to endocrine abnormalities, as described in this chapter (see also Chapter 10, Medical Management of Bone Marrow Failure in Telomere Biology Disorders and Chapter 13, Hematopoietic Stem Cell Transplantation).

Skeletal Complications

Epidemiology and Pathophysiology

The true prevalence of skeletal complications in individuals living with DC/TBDs is uncertain. Abnormal bone density/osteoporosis was reported in 3% of 86 participants in a multinational DC/TBD registry [5]. A 2017 study at the NCI reported that 27% of a cohort of 30 participants with DC/TBDs demonstrated low areal bone mineral density (aBMD) as assessed by dual energy X-ray absorptiometry (DXA) [6]. Avascular necrosis was reported as a feature in 3% of approximately 550 published cases of DC/TBDs in 2009 [7]. Fracture data are limited to case reports and thus no reliable estimates of prevalence are available. Many individuals with DC/TBDs (~75%) also have dental abnormalities such as shortened roots or taurodontism [8].

The pathophysiology underlying the adverse effects of DC/TBDs on bone density and strength are not well understood. Mesenchymal stem cell defects have been reported in individuals with DC/TBDs [9]. The cells responsible for bone formation (osteoblasts) and for teeth root development (cementoblasts) originate from mesenchymal stem cells (MSC), and could therefore be adversely affected by a defect in MSC availability,

longevity or function. Pre-clinical data further suggest that telomerase deficiency, a defining feature of DC/TBDs, specifically diminishes MSC differentiation into osteoblasts [10]. Additionally, the longevity and the maintenance of osteogenic potential of osteoblast lineage cells appears to be dependent upon telomerase [11, 12]. Interestingly, the activity of osteoclasts (cells responsible for bone resorption) does not appear to be significantly affected by the absence of telomerase [13]. Diminished bone formation without loss of bone resorption would be expected to lead to an imbalance in bone turnover that could adversely affect bone accrual during childhood and lead to bone loss in adults [14].

AVN occurs when the blood vessels supplying bone are compromised, leading to death of bone and bone marrow supplied by those vessels [15]. This results in pain, degenerative arthritis, and decreased function of the joint, and may require early joint replacement [15, 16]. Individuals with DC/TBDs and their parents should be educated at diagnosis regarding the early signs and symptoms of hip and shoulder AVN, since early conservative (restricted weightbearing, and antiresorptive medication, for example) and orthopedic surgical (core decompression, osteotomy, bone grafting) management may reduce disability and complications. In the presence of hip or shoulder pain in patients with DC/TBDs, physicians should perform a thorough physical exam that includes assessment of pain level, range of motion, limb length, and X-ray imaging of the affected area. Physicians should have a low threshold for skeletal imaging, as well as orthopedic referral in these patients. There are some data to suggest a potential role for bisphosphonates to slow progression of AVN or ameliorate AVN related pain in other conditions [17, 18] However, the efficacy remains uncertain and has not been specifically studied in patients with DC/TBDs [19].

Clinical Management

Because of the phenotypic variability in DC/TBDs, it is difficult to make generalizations about the timing of onset and severity of skeletal complications in this disease. Likewise, there are limited observational and interventional data in individuals with

DC/TBDs to guide the clinical monitoring and treatment of these complications when they arise. Recommendations must therefore be adapted from those established for other disorders affecting bone health in children and adults.

All individuals with DC/TBDs should be queried about their fracture history, including the number, location and mechanism of fracture(s), if present. Minimally traumatic fractures (typically defined as fall from standing height or less, at no more than a walking speed) of the spine, femur, humerus are especially concerning for an underlying impairment in bone health and should prompt consideration of further evaluation [20]. Patients who have had HCT are also at higher risk and should be considered for further bone health evaluation.

The clinical evaluation in patients determined to be at greater risk for impaired bone health should include assessment of dietary intake, weight-bearing physical activity, biochemical screening, and a DXA scan. The dietary assessment should include both macro- and micronutrient intake, with special attention to calcium and vitamin D. If possible, it is helpful to have this performed by a registered dietician or clinical nutritionist. Dietary education should be provided to patients to ensure they are meeting the age- and sex-specific recommended dietary allowance (RDA) for calcium and vitamin D [21]. Calcium and vitamin D supplementation can be considered in individuals who cannot meet the RDA from dietary sources alone. Individuals should be educated on and encouraged to meet the age-specific goals for physical activity [22]. Individuals with functional limitations may benefit from a physical therapy evaluation to help devise a safe physical activity plan.

Baseline biochemical screening should include a comprehensive metabolic panel, phosphorus, and 25-hydroxy vitamin D (25-OHD), at minimum. Vitamin D supplementation should be provided to achieve and maintain a serum 25-OHD level of at least 20 ng/mL (50 nmol/L), and preferably > 30 ng/mL (75 nmol/L) in patients with bone health risk factors [23]. Cholecalciferol (vitamin D3) is generally preferred, but ergocalciferol (vitamin D2) may also be used. The vitamin D dose to achieve sufficiency

will vary individually, typical regimens might include daily cholecalciferol of 1000 IU (25 mcg) to 4000 IU (100 mcg) or weekly ergocalciferol 50,000 IU (1250 mcg). Serum 25OHD levels should be monitored at least annually by the patient's health care team, more frequently in those being treated with high doses of vitamin D for severe vitamin D deficiency.

Determination of aBMD by (DXA) is an important component of the bone health evaluation. At present, there are limited data to inform the optimal timing for attaining first DXA in individuals with DC/TBDs. Clinical criteria [16] that suggest the need for DXA include a single long-bone or vertebral fragility fracture, multiple long bone fractures of any mechanism, chronic exposure to supra-physiologic steroids (6 months or greater), prolonged hypogonadism (in an adolescent or adult), and history/planned HCT. Older individuals with DC/TBDs but without corticosteroid exposure, fracture history, or hormone deficiency may be screened and monitored according to age-specific DXA scan screening guidelines.

DXA assessment should follow standard International Society for Clinical Densitometry guidelines, which are available for children and adults (Table 1) [24]. In children, aBMD should be assessed by Z-score, which provides a comparison to expected aBMD for a given age and sex. In older adults, T-scores are used to evaluate for the degree of bone loss compared to peak bone mass. However, in the case of younger adults, Z-scores may be preferable and allow for an assessment of disease specific effects on aBMD. Well validated fracture risk assessment tools may also be used in adults.

Table 1: Recommended Approach to DXA Assessment in Patients at Risk for Impaired Bone Health

Age	Standard Scan Sites	Alternative Scan Sites	Analysis Notes
3-5 years		• AP Lumbar spine	• Z-scores
5-18 years	• AP Lumbar spine • Whole body (less head)	• Proximal femur: consider in post-pubertal adolescents • Forearm: Consider when other sites cannot be assessed due to contracture, etc	• Z-scores, adjusted for size in short or tall individuals
≥ 18 years	• AP Lumbar spine • Proximal femur	• Forearm	• Z-scores: Pre-menopausal women and men <50 years • T-scores: Post-menopausal women and men ≥50 years

Recommendations taken from ISCD official positions (revised 2019). Selection of scan site requires availability of adequate reference data to generate Z and/or T scores for population of interest and DXA manufacturer. Recommended regions of interest include "L1-L4", lumbar spine; subtotal (total body less head), whole body; total hip and femoral neck, proximal femur; distal 1/3 radius, forearm.

There are limitations to DXA. aBMD by DXA is an estimate of true volumetric BMD and is subject to size artifact. Specifically, BMD is underestimated in patients with small bones and overestimated in those with large bones. This is especially relevant in children with short stature or pubertal delay, which can occur in patients with DC/TBDs. As a result, aBMD Z-scores should be adjusted for size in children with short or tall stature, using an appropriate methodology for the specific DXA platform on which the scan was obtained [25-27].

The indications for the initiation of anti-osteoporosis pharmacotherapy for the treatment or to prevent osteoporosis specific to DC/TBDs is uncertain. Therefore, the standard approach used in other conditions should be applied. For children, bisphosphonates are typically not started until after first clinically significant fragility fracture, with or without low BMD, in an at-risk patient with limited expectation of spontaneous recovery [28]. In adults, bone protective therapy can be considered according to approved Food and Drug Administration or other regulatory agency

indications. Choice of agent should be individualized based upon clinical scenario and an individualized assessment of potential toxicities of respective drugs.

Growth and Growth Hormone

Short stature is reported in 12% of cases in the literature and in approximately 20% of individuals in the UK DC/TBD registry of patients with DC/TBDs [1, 3] However, the NCI cohort notes that short stature is very rare in individuals with DC/TBDs, perhaps being more common in very severely affected patients. While the precise mechanism is unknown, short stature in patients with DC/TBDs is not due to growth hormone deficiency [4]. and growth hormone therapy is not recommended unless the patient is proven to be growth hormone deficient on formal testing.

Hypogonadism

A small number of severely affected males reported decreased sperm or testosterone production, or both, a condition known as primary hypogonadism [16, 29] Animal models have demonstrated that at least one of the DC-associated gene mutations may lead to testicular atrophy in males and decreased fertility in both males and females, but this has not been duplicated in human studies [30, 31]. Physicians should check morning testosterone, LH, and FSH levels in patients with suspected hypogonadism, and have an open dialogue about both the possibility and signs and symptoms of hypogonadism.

Interventions That Affect the Endocrine System

Androgen Therapy

Patients with DC/TBDs and bone marrow failure who are not candidates for HCT may be treated with androgens [32, 33] (see also Chapter 10, Medical Management of Bone

Marrow Failure in Telomere Biology Disorders). Low peripheral blood counts respond adequately enough in approximately 50-70% of patients with DC/TBDs treated with androgens to obviate the need for transfusion [1, 34, 35]. The duration of this effect varies from patient to patient.

The androgen medications most commonly used in DC/TBDs include oxymetholone and danazol. Androgen therapy in patients with DC/TBDs can affect the endocrine system in several ways, including decreasing thyroid binding globulin (TBG), inducing rapid linear growth as well as virilization, dyslipidemia , and changes in liver structure and function. Patients with DC/TBDs treated with androgens should be evaluated frequently for abnormal cholesterol, triglycerides, liver function, and undergo imaging to assess for liver adenomas (see Chapter 10) [36]. It should be noted that patients with DC/TBDs can be more sensitive to androgens than the general population, and therefore the dose should be adjusted accordingly [16]. It is important that individuals with DC/TBDs and their families be aware of possible side effects of androgen therapy before initiation of treatment [37].

Thyroid Binding Globulin

Treatment with androgens can cause a significant decrease in liver synthesis of TBG without actually resulting in a change in thyroid function [33]. Despite a low TBG level, patients with normal thyroid glands are biochemically euthyroid with a normal serum free thyroxine (T4) and thyroid stimulating hormone (TSH) levels, and thus do not experience any clinically noticeable adverse effects [37].

Growth

Androgens stimulate osteoblasts and the production of bone matrix. As a result, pre-pubertal children receiving androgen therapy can experience premature accelerated linear growth [38, 39]. Premature skeletal maturation, with fusion of epiphyseal plates and reduced final adult height has been reported, but this effect is relatively rare, especially in patients who received androgen therapy prior to pubertal development [33,

38, 40]. Pediatric patients should undergo a baseline bone age X-ray evaluation of the left hand before initiation of androgen therapy and every 12 months while undergoing androgen treatment, until reaching final adult height. Patients receiving androgen therapy should avoid simultaneous use of growth factors like G-CSF or erythropoietin, as splenic and hepatic peliosis and splenic rupture have been reported in association with concomitant combined use [35, 38, 41].

Masculinization and Behavior Changes

Androgen therapy may lead to virilization and behavior changes, including mood swings and aggression in both female and male patients [16, 33, 42]. Females receiving androgens can develop deepening of the voice and hirsutism (excessive hair growth), while males report priapism (persistent and painful erection of the penis) [33]. The likelihood of onset and degree of virilization are directly proportional to androgen dose and duration of use [38, 42].

Lipid and Liver Abnormalities

Patients with DC/TBDs receiving androgen therapy can develop dyslipidemia and impaired liver function [16, 43]. Total cholesterol, triglycerides, and LDL (low-density lipoprotein) may be elevated, while HDL (high density lipoprotein) levels may be abnormally low [33, 43]. There is no known dose-response relationship between androgens and lipid abnormalities, but cessation of androgen therapy results in a return to pre-treatment lipid levels within a few months [38]. Liver structure and function should be evaluated with imaging at baseline prior to initiation of treatment and at regular intervals during androgen therapy, as liver adenomas and carcinomas have been observed in patients on this regimen [16].

Allogeneic Hematopoietic Cell Transplantation (HCT)

HCT is the only curative treatment for bone marrow failure in DC/TBDs; however, its conditioning regimen is associated with significant toxicity (see Chapter 13,

Hematopoietic Stem Cell Transplantation) [44, 45]. With the development of new, less toxic conditioning regimens, HCT may become a more successful treatment modality for patients with DC/TBDs [46].

HCT survivors and their physicians should be aware of an increased risk of multiple endocrinopathies, including hypothyroidism, growth failure, hypogonadism, osteoporosis, adrenal dysfunction, anterior pituitary disorders, diabetes mellitus and dyslipidemia [47]. Post-HCT screening and follow-up should be tailored to the specific conditioning regimen received by each patient.

Diabetes Mellitus

HCT survivors should have routine screening for diabetes mellitus, given higher likelihood for insulin resistance compared to healthy counterparts [47-49]. Steroid use, total body irradiation, and graft versus host disease all increase the likelihood of future risk for diabetes mellitus [47]. Therefore, HCT survivors benefit from an annual screen for insulin resistance and receive pharmacotherapy if they demonstrate glucose intolerance and diabetes mellitus [47].

Hypogonadism After HCT

Patients with DC/TBDs can experience hypogonadism as a result of alkylating chemotherapy exposure with HCT [16, 29, 46]. In males, hypogonadism can be due to Seratoli cell damage and decreased sperm production, and/or Leydig cell dysfunction resulting in low testosterone production [47]. In females, HCT can result in primary ovarian insufficiency consisting of either acute ovarian failure or premature menopause [50-52]. Reduced estrogen production leads to low libido, and vaginal changes [47]. In both males and females, clinicians and patients should have pertinent discussions regarding the impact of HCT on fertility and preservation options pre-HCT such as sperm banking in males and ovarian cryopreservation, as well as post-transplant in vitro fertilization [47, 53]. Therapy for hypogonadism should be tailored to individual patient

goals, as well as to preventing and treating other complications such as bone loss and cardiometabolic impact of sex hormone deficiency [47].

Osteoporosis

As introduced above, based upon data from other hematologic disorders treated with HCT [54-56], it is likely that patients with DC/TBDs who have undergone HCT are at increased risk of developing low BMD and osteoporosis. Steroids (both corticosteroids such as prednisone, and androgens), hypogonadism, direct damage to osteoprogenitor cells, hyperthyroidism, immobility, and calcium or vitamin D deficiency, all increase the risk of osteoporosis in HCT recipients [44]. Clinical guidelines for the management of bone health following HCT for hematologic disorders have been published for both children and adults [57, 58]. Dietary intake and physical activity should be optimized as described above. Biochemical testing is recommended at least every 6 months in the first year, and then annually thereafter. DXA is recommended prior to or at least within 3 months following transplant, with a follow up scan 12-months later. The need/timing of subsequent testing is based upon clinical course, including BMD status. Annual vertebral fracture assessment by lateral spine X-ray or DXA should be performed in patients on chronic supraphysiologic glucocorticoid doses or those with back pain. High quality clinical trial data to guide the use of bone protective agents in patients following HCT are lacking. However, expert opinion guidance has been published [58].

AVN has been reported in 4-19% of allogeneic HCT patients [47]. Given the increased risk of AVN, post-transplant patients should be educated about its signs and symptoms. Physicians should have a high index of suspicion for this complication following onset of new joint or limb pain. Patients who have received total body irradiation, those who have received corticosteroids, and those of advanced age are at particularly high risk of AVN, and should be evaluated accordingly [47, 57, 58]. The treatment approach to HCT related AVN does not differ from the standard approach described above.

Adrenal Insufficiency

All patients receiving treatment with supra-physiological doses of glucocorticoids (> 7.5 mg/d) can experience suppression of the hypothalamic pituitary axis or primary adrenal insufficiency with reduced function of the adrenal gland. These can result in fatigue and weakness [47]. Adrenal insufficiency is assessed using an ACTH (adrenocorticotropic hormone) stimulation test, but providers should be aware that even treatment with low doses of steroids can result in an abnormal stimulation test result. As a result, consultation with an endocrinologist is recommended to help address concerns and direct treatment for adrenal insufficiency in DC/TBDs patients following HCT [47].

Growth Hormone Deficiency

GH deficiency can occur following fractionated doses of 12-18 Gy when given as total body irradiation in the context of HCT [59]. Growth hormone deficiency should be suspected when there is linear growth failure (height trajectory crossing to lower percentile lines) or lack of growth acceleration during puberty, after ruling out hypogonadism, hypothyroidism, inadequate nutritional intake, or excess glucocorticoid exposure. Determination of upper to lower segment ratio using sitting height or lower segment is helpful to rule out poor spinal growth after radiation [60]. Insulin-like growth factor-I (IGF-I) levels are not always low in the context of radiation-induced GH deficiency; they should not be used to screen patients at risk [61]. When GH deficiency is suspected, a referral to a pediatric endocrinologist is indicated for further evaluation with stimulation testing.

Replacement with recombinant human GH (hGH) results in significant improvement in height in children with confirmed GH deficiency, but patients may not achieve their genetic potential due to other factors such as spinal [60] or total body irradiation [62] or scoliosis. Treatment with hGH may improve cardiovascular risk factors such as dyslipidemia and quality of life, but studies are lacking in children and results are variable and limited [63, 64].

Thyroid Disorders

Primary hypothyroidism is the most common endocrine abnormality following HCT [65]. Age under ten at the time of transplant and conditioning regimen involving total body irradiation, or chemotherapy with busulfan-cyclophosphamide, and presence of hematologic malignancy all further increase the risk of hypothyroidism among HCT survivors [47]. Therefore, individuals should have routine surveillance screens with annual thyroid function testing. Some patients may experience subclinical hypothyroidism which may resolve without any treatment, while others may require pharmacological intervention to establish biochemical euthyroidism [47].

Hyperthyroidism is rarely seen in HCT survivors, and far less common than primary hypothyroidism [47]. Patients with hyperthyroidism may experience anxiety, increased sweating, palpitations, weight loss, or diarrhea due to elevated thyroid hormone levels [47]. Symptomatic patients should have TSH, T3 and free thyroxine levels drawn, as well as assessment for thyroid antibodies [47]. Consultation with an endocrinologist is warranted for management and treatment of a symptomatic patient with hyperthyroidism.

While DC/TBDs are not associated with increased risk of thyroid cancer, radiation exposure as part of HCT conditioning regimen can lead to increased risk for thyroid nodules and thyroid malignancy in HCT survivors [47, 65]. Therefore, HCT survivors with a history of total body irradiation exposure benefit from surveillance imaging with ultrasound and identified nodules should be further evaluated with fine needle aspiration biopsy if the nodule meets threshold and criteria for biopsy [66]. Survivors with confirmed diagnosis of differentiated thyroid carcinoma by fine needle aspiration biopsy should have complete pre-operative staging of the neck with an ultrasound to assess for cervical lymph node metastasis and be referred to experienced surgeon in thyroid surgery for appropriate surgical management of the patient. Post-surgical evaluation and assessment needed for additional treatment such as radioactive iodine should be based on post-operative risk classification according to the pediatric

American Thyroid Association guidelines [67]. Patients with benign thyroid nodules should continue to have regular surveillance and undergo biopsy as needed based on imaging determination.

Table 2. Summary of reported endocrine and skeletal abnormalities in DC. Prevalences estimated from the literature and NCI DC/TBD cohort [6].

Features	Estimated Prevalence
Short stature	20%
Hypogonadism/undescended testes	6%
Growth hormone deficiency	1 patient
Low bone mineral density	3-27%
Avascular necrosis	3%
Fracture	Not systematically reported

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Chapter 23

Radiation and Telomere Biology Disorders

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Introduction

Individuals with dyskeratosis congenita (DC) and related telomere biology disorders (TBDs) have very short telomeres for their age caused by pathogenic germline variants mutations in genes essential in telomere maintenance (see also Chapter 2, Why Telomeres Matter and Chapter 4, The Genetics of Dyskeratosis Congenita and Telomere Biology Disorders) [1-3]. Telomeres are long repeats of DNA nucleotides [(TTAGGG)$_n$] and a protein complex at chromosome ends essential for chromosomal stability. In general, the telomeric DNA repeats are sensitive to DNA damage caused by radiation and oxidative stress [4-6]. Since

individuals with DC/TBDs have very short telomeres from birth, they may also be more sensitive to the effects of both ionizing and non-ionizing ultraviolet radiation [7, 8].

When therapeutic or interventional radiation [1] is being considered for patients with DC/TBDs, precautions should be taken to prevent or minimize harm. It should be noted that there are very little data on radiation effects in these individuals. The radiation risk from clinically indicated medical imaging is extremely low. Diagnostic exams should be performed if the information obtained from medical imaging is important for patient care. This chapter reviews the types of radiation that may be of clinical importance in DC/TBDs.

Types of Radiation

Ionizing radiation is radiation that can pass through the body. It carries enough energy to liberate electrons from atoms, creating ions, which can in turn damage DNA and cause cell death. Examples include X-rays and gamma rays used in medical imaging and certain interventional or therapeutic procedures.

Diagnostic radiation, such as x-rays used in bone density scans, radiography, diagnostic fluoroscopy, computed tomography (CT), and gamma rays used in single photon emission tomography (SPECT) and positron emission tomography (PET), typically use very low levels of ionizing radiation. Interventional radiation is sometimes used in interventional radiology, cardiology, and operating room procedures, and uses moderate levels of X-ray ionizing radiation. Therapeutic radiation involves much higher doses of ionizing radiation, including X-rays and gamma rays, and is designed to treat cancer or prepare a patient for hematopoietic cell transplant (HCT).

Ultraviolet radiation consists of light rays slightly more energetic than the color violet and can excite electrons to move to a higher energy state. However, such radiation does not carry enough energy to produce charged ions, so ultraviolet radiation is considered "non-ionizing". For protection from ultraviolet radiation, individuals with DC/TBDs need to avoid tanning beds and take precautions to minimize sun exposure as much as reasonable (see also Chapter 6, Dermatologic Manifestations) [1].

Ultrasounds (sonograms) and magnetic resonance imaging (MRI) do not use ionizing radiation, have no known ill effects specific to patients with DC/TBDs, and are not discussed in this chapter.

Effects of Radiation

There are two types of radiation effects: tissue reactions and stochastic effects.

Tissue Reactions

Tissue reactions are defined as those that cause cell death or an injury in populations of cells. The type of tissue reaction is based on the dose of radiation, sensitivity of the specific tissue type exposed, and the individual's underlying sensitivity to radiation. There is a threshold dose to producing a tissue reaction such that there are doses below a certain level in which there is no reaction, and above which the harm to tissue reaction increases as the dose increases [9].

Therapeutic radiation (generally thought of as higher-dose radiation) is used to create a tissue reaction in order to kill cancer cells. It is also used as part of some hematopoietic cell transplantation (HCT, see also Chapter 13, Hematopoietic Stem Cell Transplantation) preparation regimens to eliminate the patient's bone marrow cells so they can receive donor cells. Tissue reactions can also occur in healthy tissue near the area of the body targeted by therapeutic radiation. Very rarely, tissue reactions occur

when using moderate dose interventional radiation as part of a procedure that requires X-rays to see inside the body.

Stochastic Effects

Stochastic (random) effects generally refer to the potential occurrence of cancer in an individual who received radiation therapy, or after radiation exposure from medical imaging or interventional imaging or procedures [9]. Stochastic effects of radiation might occur years after exposure to ionizing radiation. They can be thought of as the long-term consequences of radiation exposure. It is often difficult to know whether stochastic effects are caused by radiation, lifestyle choices, or from natural biological or environmental causes. The risk of cancer is believed to increase with increasing radiation dose, although this has not yet been definitively proven for effective doses less than 100 mSv [9].

Therapeutic Radiation

Therapeutic radiation is high dose radiation used to create controlled tissue reactions as part of a clinical protocol. Examples where this type of radiation is used include cancer radiotherapy and total body irradiation for HCT.

Patients with DC/TBDs appear to have a lower threshold for tissue reactions than other patients when exposed to therapeutic radiation [1, 8, 10, 11]. However, detailed studies of ionizing radiation in DC/TBDs have not been conducted to better define these lower thresholds. Even though tissue sparing techniques like proton therapy show promise [11], more studies are needed to better determine optimal use of therapeutic radiation for cancer in patients with DC/TBDs [1].

HCT protocols for patients with DC/TBDs are being developed that use reduced intensity total body irradiation, or none at all, and have improved clinical outcomes

compared with full dose irradiation procedures (see also Chapter 13, Hematopoietic Stem Cell Transplantation) [12-14].

Interventional Radiation

Interventional radiation is moderate dose radiation used in minimally invasive procedures to see inside the body while performing certain treatments or surgeries. For complex cases, there is a chance of exceeding a threshold dose that can cause unintentional tissue reactions [15].

Based on what has been observed from therapeutic radiation for patients with DC/TBD, there is reason to believe that tissue reactions might occur at a lower threshold than in the general population for interventional radiation. Examples of interventional radiation include certain cardiology, urology, and angiography procedures.

It is important to know if X-rays are used for these types of clinical procedures, and to know where X-rays enter the body. This information will help patients identify potential rashes from the interventional radiation exposure, which is often the first sign of a tissue reaction. Patients should report any rashes of concern to their primary provider.

Diagnostic Radiation

Diagnostic radiation (low dose radiation) is used for medical imaging. This type of radiation includes X-rays used in mammography, bone density scans, radiography, diagnostic fluoroscopy, computed tomography (CT), and gamma rays used in nuclear medicine (NM) studies, including single photon emission computed tomography (SPECT) and positron emission tomography (PET). The doses used in diagnostic procedures are far too low to cause tissue reactions but could cause stochastic effects in patient populations.

Diagnostic radiation doses can be compared to natural background radiation (see Table 1). For diagnostic imaging, the chances of stochastic effects are so low that they cannot be measured in an individual patient, but are estimated for patient populations. In fact, many radiation safety professional organizations now clearly state that there could very well be no effect for effective doses below 100 mSv [16, 17]. However, the medical imaging community continues to assume there is some risk from radiation at these lower doses, to be conservatively safe.

Although patients with DC/TBDs might be more sensitive to stochastic effects from ionizing radiation than the general population, the doses required for diagnostic purposes are very low. Clinically indicated exams should be performed when needed. One must always weigh the benefit of proceeding with care using information from imaging against the very low, and possibly non-existent, harm from the imaging exam, as well as the harm in proceeding with care without appropriate imaging.

Summary

Radiation exposure of individuals with DC/TBDs should be managed proactively. Patients with DC/TBDs are more sensitive to therapeutic radiation (high dose radiation) than the general population, but the degree of this sensitivity has not been established. Clinically indicated interventional procedures (interventional radiation) and medical imaging (low dose radiation) should be performed when clinically appropriate for optimal patient care since the benefit of performing these clinically indicated procedures or exams far outweighs the potential harm from radiation effects.

Table 1. Patient dose from diagnostic X-ray exams [18, 19] compared to natural background radiation to provide context. Background radiation dose varies from 1 to 10 mSv per year [20] depending on where one lives. This table compares medical imaging effective doses to the natural background radiation level of 4 mSv per year.

Diagnostic Exam	X-ray Dose	Amount of Time to Receive Similar Dose from Natural Background Radiation
Knee X-ray, Dental X-ray	0.005 mSv	11 hrs
Dental panoramic X-ray	0.01 mSv	22 hrs
Bone Density Scan	0.015 mSv	1.5 days
Chest X-ray	0.02 mSv	2 days
Lumbar spine X-ray	1.5 mSv	4.5 months
Head CT	2 mSv	6 months
Chest CT	7 mSv	21 months
Abdomen/Pelvis CT	14 mSv	42 months

Abbreviations: millisievert (mSv), a measure of the effective dose of radiation. Effective dose is used to estimate stochastic risk in patient populations.

Table 2. Summary of Chapter Terminology

Types of Radiation	
Ultraviolet Radiation	- Ultraviolet (UV) light (UV radiation) is invisible to the human eye. It is not energetic enough to ionize atoms and is therefore considered non-ionizing radiation. It can, however, damage overexposed skin. - A beneficial effect of UV radiation exposure is vitamin D production in the body. - UV radiation comes from the sun and is filtered by the atmosphere. UV radiation is also produced by electrical arcs (like welding), tanning lamps, and blacklights.

Ionizing Radiation	- Ionizing radiation is powerful enough to remove electrons from atoms (ionize atoms), which can damage DNA and cause cell death. - Ionizing radiation can cause tissue reactions and stochastic effects (see below). - Most cosmic ionizing radiation is absorbed by the atmosphere. - Diagnostic, interventional, and therapeutic radiation in medicine eases pain and saves lives.
Patient Exposure to Ionizing Radiation	
Background Radiation	- Low dose radiation. - Background radiation comes from the sky, the soil, and what we eat. We naturally live in a bath of radiation.
Diagnostic Radiation	- Low dose radiation. - Medical uses include X-rays and gamma rays used for medical imaging. - Ionizing radiation is used to see inside the body to aid with important diagnosis and monitor function. - Stochastic effects might occur from diagnostic radiation. - Patients with DC/TBDs might be more sensitive to stochastic effects than the general population, but not so sensitive that clinicians should avoid clinically appropriate medical imaging.

Interventional Radiation	● Moderate dose radiation. ● Ionizing radiation is used in minimally invasive procedures to see inside the body while performing treatments or surgeries. ● Very rarely, radiation levels exceed a threshold where tissue reactions occur. ● Based on what has been observed from therapeutic radiation, there is reason to believe that tissue reactions might occur at a lower threshold for patients with DC/TBDs than in the general population for interventional radiation.
Therapeutic Radiation	● High dose radiation. ● The purpose of therapeutic radiation is to create controlled tissue reactions as part of a clinical protocol. ● Examples where this type of radiation is used include cancer radiotherapy for cancer treatment and total body irradiation for hematopoietic stem cell transplants. ● Case studies of cancer therapy in patients with DC/TBDs have shown that tissue reactions occur in non-cancerous tissue at lower doses than the general population. Total body irradiation is more toxic to these patients, which has led to the development of reduced intensity or elimination of radiation in conditioning in hematopoietic cell transplant protocols.
Radiation Effects	
Stochastic Effects	● Effects that can occur by chance, especially malignancies or genetic mutations. The probability of occurrence increases with radiation dose, but the severity is independent of dose. ● There is no threshold; severity of effect is not dose-dependent.

Tissue Reactions	• Injury in populations of cells or cell death.
	• There is a threshold dose. Once above the threshold, the severity of the reaction increases as dose is increased.

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Chapter 24

Neuropsychiatric Complications

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Introduction

People living with DC have a higher risk for structural brain abnormalities which may manifest as neurodevelopmental disorders, neuromotor impairment, and psychiatric diagnoses when compared with the general population. Thus, careful screening for these manifestations is indicated. Further study of this population has the potential to continue to yield significant insights into the pathobiological connections between telomere biology and development of neuropsychiatric conditions.

Telomeres and Psychiatric Disorders

There is significant research interest in the role of telomeres in psychiatric disorders. Cross-sectional studies have identified associations between shorter blood or buccal cell telomere length and psychiatric diagnoses such as major depressive disorder [1, 2], bipolar disorder [2], schizophrenia [3], and post-traumatic stress disorder (PTSD) in adulthood following childhood trauma [4]. One study found reduced levels of lymphocyte telomerase in individuals with schizophrenia [5]. Shorter germline telomeres were also noted in subjects with significant psychosocial stress, such as adult caregivers of the chronically ill [6], women who have experienced domestic violence [7], and in chronically institutionalized children from Romania [8]. There is some suggestion that cumulative number of stressors may have a differential impact on later telomere length [9]. Stress-induced hypothalamic-pituitary-adrenal axis activation may play a role in mediating the relationship between stressors and shortened telomeres [10], as cortisol is known to reduce telomerase activity.

Most of the above studies evaluated telomere length in peripheral blood leukocytes, which may not correlate with telomere length in other cells. A study of telomere length in cortical neurons showed no difference between patients with major depressive disorder and control subjects [11], while another study looking at cerebellar neurons demonstrated no link between telomere length and serious psychiatric illness [12]. It is important to note that in studies demonstrating telomere length association between cases and controls, the differences may be statistically significant, but are still relatively small when control telomeres are compared to the markedly short telomeres of dyskeratosis congenita (DC). Thus, it remains unclear whether short telomeres predispose patients to develop certain neuropsychiatric conditions, or that telomere shortening is a downstream consequence of the physical effects of psychiatric symptoms and stress [13]. Alternatively, telomere shortening in the face of neuropsychiatric conditions may be expressions of a common biological insult.

Neuropsychiatric Disorders and Dyskeratosis Congenita

There are limited data on the relationship between DC and neuropsychiatric conditions. Most individuals with DC have normal intelligence and achieve normal developmental motor milestones, although severely affected individuals may not. Developmental delay is present in two subtypes of DC: Hoyeraal Hreidarsson (HH) [14, 15] and Revesz Syndrome [15, 16]. Like classic DC, these disorders are characterized by the mucocutaneous triad described in Chapter 3, Diagnosing Telomere Biology Disorders. In addition, Revesz syndrome is remarkable for bilateral exudative retinopathy and intracranial calcifications. Immunodeficiency is seen in HH, and changes reported in neuroimaging, including cerebellar hypoplasia or atrophy, small brainstem, thin corpus callosum, and cerebral calcifications, may be confused with TORCH syndrome, caused by a group of neonatally acquired infections (Toxoplasmosis, Other, Rubella, Cytomegalovirus and Herpes infections).

There are relatively scant data about psychiatric illness in individuals with DC. Two case reports describe schizophrenia in these patients [17, 18] and a recent case describes an adult presenting with mood (mania) and psychotic symptoms [19]. In 2012, a preliminary study of six pediatric and eight adult patients with DC or DC-like conditions demonstrated a relatively high incidence of some form of neuropsychiatric disorder [20]. Participants had a wide variety of psychiatric concerns, but mood disorders were the most common. Neurodevelopmental diagnoses such as attention deficit hyperactivity disorder (ADHD), intellectual disabilities, learning disabilities, or autism spectrum disorder were also very common in this sample, with half of pediatric subjects and a quarter of adults carrying at least one of these diagnoses (Table 1) . A more recent examination of 44 participants with telomere biology disorders (26 children, 18 adults) (31 DC, 12 HH, and 1 Revesz syndrome) included structural brain magnetic resonance imaging (MRI) and showed 25/44 (57%) patients had one or more structural brain abnormality or variant [20]. While this expanded longitudinal study included 10 patients previously reported [21], the data continue to support increased neuropsychiatric findings with 21 patients (48%) having neurodevelopmental disorders or psychomotor

abnormality and 12 patients (27%) having psychiatric diagnoses, including depression and/or anxiety disorders. In this study, shorter lymphocyte telomere length was associated with more brain MRI findings/neurodevelopmental abnormalities and persons with autosomal recessive or X-linked telomere brain disorders had more neurologic findings than those with autosomal dominant disease [20] (Table 1).

Table 1. CNS findings by age and mode of inheritance in patients with TBDs. Values in parentheses represent percentages. (Derived from Bhala et al, *Neurol Genet.* 2019 [20])

	X-linked or autosomal recessive		*TINF2*		Autosomal dominant		Gene unknown		All TBDs		
	Children n=11	Adults n=9	Children n=6	Adults n=3	Children n=4	Adults n=5	Children n=4	Adults n=2	Children n=26	Adults n=18	Total n=44
MRI Abnormalities	9 (82)	5 (56)	4 (67)	0	2 (50)	3 (60)	1 (25)	1 (50)	16 (62)	9 (50)	25 (57)
Cerebellar hypoplasia or atrophy	8 (73)	4 (44)	0	0	2 (50)	1 (20)	1 (25)	1 (50)	11 (42)	6 (33)	17 (39)
Cerebral atrophy	2 (18)	3 (33)	1 (17)	0	0	2 (40)	0	0	3 (12)	5 (28)	8 (18)
Periventricular cysts	2 (18)	1 (11)	3 (50)	0	0	1 (20)	1 (25)	0	6 (23)	2 (11)	8 (18)
Corpus callosum abnormalities	3 (27)	1 (11)	3 (50)	0	0	0	0	0	6 (23)	1 (6)	7 (16)
Small pons	2 (18)	0	1 (17)	0	2 (50)	0	0	0	5 (19)	0	5 (11)
Neurological	10 (90)	4 (44)	4 (67)	0	2 (50)	0	1 (25)	0	17 (65)	4 (22)	21 (48)
Neurodevelopmental disorder	10 (90)	4 (44)	3 (50)	0	2 (50)	0	0	0	15 (58)	4 (22)	19 (43)
Neuro-motor	8 (73)	2 (22)	3 (50)	0	2 (50)	0	1 (25)	0	14 (54)	2 (11)	16 (36)
Psychiatric	1 (9)	5 (56)	1 (17)	1 (33)	1 (25)	2 (40)	1 (25)	0	4 (15)	8 (44)	12 (27)
Depression, anxiety, OCD, ADHD, bipolar disorder	1 (9)	5 (56)	1 (17)	1 (33)	1 (25)	1 (20)	1 (25)	0	4 (15)	7 (39)	11 (25)
Autism	1 (9)	1 (11)	0	0	0	0	0	0	1 (4)	1 (6)	2 (5)
Substance abuse	0	1 (11)	0	0	0	1 (20)	0	0	0	2 (11)	2 (5)
Microcephaly	7 (64)	3 (33)	3 (50)	0	2 (5)	0 (0)	1 (25)	1 (50)	13 (50)	4 (22)	17 (39)

It is worth noting that common treatments for DC, including androgen therapy and preparatory regimens for bone marrow and stem cell transplant, can precipitate or

exacerbate psychiatric illness. As discussed more fully in Chapter 29, Navigating Telomere Biology Disorders, psychosocial sequelae of DC could also be associated with developing psychiatric problems. Living with a chronic illness that predisposes to the development of various cancers places additional psychological burdens on patients with DC and their families. Patient concerns arising from timing of diagnosis disclosure and management of aggressive treatments, such as bone marrow transplant, may initiate or aggravate pre-existing psychiatric symptoms.

Recommendations for Patients

Neurologic and psychiatric symptoms, as well as structural brain abnormalities, are common in telomere biology disorders. Comprehensive clinical care for patients with DC should include careful neurologic, neuropsychological and psychiatric assessments and consider baseline brain MRIs for early detection and appropriate specialty referral for brain-related findings. The relatively frequent finding of intellectual disabilities, autism spectrum and other learning disorders in this patient population suggests a need to routinely monitor children with DC for problems in academic performance and achieving developmental milestones. For those patients with identified concerns, early neuropsychological assessment and close collaboration with support at the child's school can help guide academic and therapeutic interventions. Specifically, speech and language, as well as occupational and physical therapy referrals may be indicated. In addition, the physiological and psychological impact of a chronic condition such as DC and its treatments can magnify underlying risks for development of psychiatric illness. Providers caring for DC individuals should include, at a minimum, a check-in about emotional symptoms at each routine visit. A referral list of local mental health providers should be maintained for patients who would benefit from further psychological and/or psychiatric evaluation or treatment.

Future Directions

Patients with DC may be a key population in which to study potential links between telomere biology and brain disorders as intact telomeres are clearly important for embryonic and adult neurogenesis and seem important in brain development though their specific roles are unknown. Longitudinal studies could help clarify the association between telomere shortening and psychiatric illness. Genotype-phenotype correlations between genes variants in DC and neuropsychiatric disorders may also yield important information on the contribution of these genes to neurodevelopment.

Acknowledgements

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Chapter 25

Routine Healthcare for Children with Telomere Biology Disorders

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Introduction

A multidisciplinary and age-based approach to routine health care and screening is essential for all children suspected or confirmed to have telomere biology disorders (TBD) (see also Chapter 3, Diagnosing Telomere Biology Disorders). Individuals confirmed to have a TBD based on presence of known pathogenic variants in TBD-associated genes, very short (<<1%ile) lymphocyte telomere lengths, and clinical features consistent with the disease are at high risk for development of TBD clinical manifestations and require intensive routine screening. This screening may include regular visits with physicians from multiple subspecialties, peripheral blood laboratory studies, organ assessments by imaging

and functional testing, and biopsies for mucosal and bone marrow abnormalities that cannot be assessed by less invasive means.

Less intensive screening regimens that omit routine use of certain invasive tests requiring anesthesia and require fewer subspecialty evaluations in the absence of relevant symptoms may be appropriate for certain individuals with TBDs. Examples of such individuals, herein referred to as having moderate risk, include:

- Individuals with limited features of TBD who are found to have a variant of uncertain significance in a TBD-associated gene on genetic testing [1] and who lack very short lymphocyte telomeres
- Individuals who have short, but not very short, lymphocyte telomeres (1-10%ile), limited physical features that may be associated with TBD, but no pathogenic variants or variants of uncertain significance in genes associated with TBD
- Individuals with heterozygous pathogenic gene variants (*e.g.*, certain variants in *RTEL1*) that are associated with later-onset TBD disease features (*e.g.*, pulmonary fibrosis) but are not clearly associated with disease features in the pediatric age range [2, 3].

A final category of pediatric patients with TBD that require a unique approach to routine care and screening includes those who have previously undergone hematopoietic cell transplantation (HCT). The approach to routine care and screening in post-HCT patients must incorporate distinct hematologic and immunologic screening, as well as careful attention to organ dysfunction and cancer risk that may be exacerbated by HCT complications related to chemotherapy toxicity and graft versus host disease [4] (see also Chapter 13, Hematopoietic Stem Cell Transplantation). In this chapter we provide guidance for

an age-based approach to routine care and screening for pediatric patients with TBDs, stratified according to these three distinct categories of patients.

Coordination of Care: Medical Home

To ensure that all recommended routine health screening is performed, we recommend that pediatric individuals with TBD establish care with a provider willing to serve as their medical home [5]. This provider is responsible for coordinating care among involved subspecialists and for maintaining a summary detailing status of screening and individualized medical needs. Once a medical home is established, individuals should be seen by the physician serving as the medical home at least every 6 months, with more frequent visits indicated for individuals with significant medical issues requiring care coordination.

The medical home function may be performed by providers with distinct expertise backgrounds including pediatric hematologist/oncologists, transplant physicians (particularly for post-HCT patients), geneticists, immunologists, gastroenterologists, or general pediatricians dedicated to patients with complex care needs. For individuals who live in communities lacking specific providers with extensive expertise in the care of patients with TBDs, we recommend that the local physician serving as the medical home partner with a regional or national specialist in caring for the patient. In situations where the medical home function is split between a local physician and a regional TBD expert, we recommend that the every 6 month follow-up visits be split between the local and regional providers such that each sees the patients at least once per year.

Hematologic Screening

Peripheral blood counts and bone marrow screening in patients with TBDs are used to detect both the onset of bone marrow failure (BMF) as well as evidence of progression to myelodysplastic syndrome (MDS). Retrospective cohort data suggest that pediatric patients with TBD less than 10 years of age are at very low risk of developing MDS, while MDS risk increases through the adolescent and young adult age periods [6]. In contrast, BMF can develop in individuals with TBD at any age. Pediatric patients should be seen by a pediatric hematologist/oncologist or HCT physician at least yearly for hematologic monitoring.

Peripheral blood count monitoring

Patients with either confirmed high risk TBD or suspected/moderate risk TBD (as defined above) who have not received HCT should undergo screening by complete blood count (CBC) with differential and absolute reticulocyte count at least every 6 months. In patients who develop cytopenias in the red blood cell, neutrophil, or platelet lineages, or have known bone marrow abnormalities, CBC screening should be performed at least every 3 to 4 months. In post-HCT patients, CBC monitoring should adhere to institutional transplant guidelines within the first 3 years post-HCT. CBC screening can be performed yearly in long-term post-HCT patients with normal blood counts, full donor chimerism and no other severe non-hematologic complications. However, onset of non-hematologic TBD manifestations including liver and pulmonary dysfunction or bleeding due to vascular anomalies should warrant CBC screening at least every 3 to 6 months, as even with healthy graft function post-HCT, severe cytopenias can develop in these patients.

Bone marrow monitoring

In all patients newly confirmed to have high risk TBD, we recommend obtaining a baseline CBC and a screening bone marrow (BM) biopsy and aspirate. These BM studies should include:

- Assessment of morphologic dysplasia
- Assessment of blasts by morphology, flow cytometry, and immunostains
- Iron stain to assess for ringed sideroblasts,
- Reticulin stain to assess for fibrosis
- Cytogenetic analysis by G-banding
- Fluorescence *in situ* hybridization (FISH) to detect translocations or copy number changes involving chromosomes 5q, 7/7q, 8 and 20q
- Next generation sequencing panel designed to detect somatic mutations commonly associated with MDS.

For high risk patients with abnormalities detected on bone marrow screening, including hypocellularity, dysplasia, or any evidence of somatic genetic alterations, or for patients with blood count abnormalities including cytopenias or elevated MCV, repeat BM screening should be performed at least yearly. For patients less than 10 years of age with a normal initial screening bone marrow assessment and normal peripheral blood counts (including normal MCV), due to the very low risk of MDS in this population and the low likelihood of severe BM failure occurring in patients with normal blood counts, follow-up bone marrow studies may be deferred until 10 years of age as long as blood counts remain normal. Due to the increasing risk of MDS in the second decade of life in patients with confirmed high-risk TBD, even patients with normal blood counts and a history of normal bone marrow evaluation(s) should begin yearly screening BM aspirates/biopsies after age 10 to screen for evidence of clonal hematopoiesis serving as a harbinger for MDS.

For patients in the moderate risk category (those with suspected but not confirmed TBD and those with genetic variants not associated with severe pediatric onset manifestations), we still recommend an initial screening BM aspirate and biopsy, which in addition to defining hematologic risk can be an important diagnostic test to provide additive evidence for a TBD diagnosis. However, if initial BM studies as well as ongoing CBC evaluations are normal for patients in this category, further BM studies can be deferred throughout the pediatric age range unless concerning CBC or clinical changes

arise, or more definitive evidence is found to confirm a high risk TBD with pediatric-onset manifestations. As is the case for patients in the high risk category, moderate risk patients found to have cytopenias, an elevated MCV, or abnormal findings on bone marrow screening should undergo annual BM evaluations.

Finally for patients who are post-HCT, routine BM evaluations are not generally recommended. Exceptions include patients who underwent HCT due to development of MDS or leukemia, in whom post-HCT BM monitoring for hematologic relapse may be part of institutional standards of practice, or patients with recurrent onset of severe cytopenias after HCT who are suspected to have developed graft failure.

Immunologic Screening and Approach to Immunizations

While some individuals with TBD have intact adaptive immunity, many patients may suffer from impaired immune function ranging from early onset severe combined immunodeficiency or immune dysregulation to more subtle common variable immune deficiency [7]. All patients in the high risk and moderate risk categories for TBD should undergo screening immune function assessment at diagnosis, including:

- Flow cytometry-based enumeration of immune cell subsets that includes:
    - CD3, CD4, CD8 T cells, along with memory and naïve T cell fractions
    - CD19/CD20 B cells
    - NK cells
- Immune globulin (Ig) quantification including IgA, IgM, and IgG
- Vaccine titers including responses to tetanus and pneumococcal vaccines

Patients with significant abnormalities detected on this initial screening should establish care with a local or regional immunologist, with whom yearly follow-up is recommended. Because immune function may be dynamic and exhibit deterioration with age in patients with TBD, patients with normal initial immunologic screening should

undergo repeat screening with onset of increased sinopulmonary or atypical infections, or with onset of moderate to severe BMF.

Patients with normal initial immunology screening should receive inactivated vaccines per standard pediatric schedules such as the United States Center for Disease Control (CDC) schedule (https://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html). Receiving vaccination against human papilloma virus (HPV) as per CDC schedules is of particular importance to individuals with TBD, given the implication of HPV in some head/neck, urogenital, and anal cancers [8, 9]. However, if patients demonstrate inadequate quantitative immune function or inadequate titers following tetanus and pneumococcal vaccination, consultation with an immunologist regarding approach to vaccination is recommended, and live virus vaccinations including varicella and measles/mumps/rubella (MMR) vaccines should be deferred until immunology consultation has occurred.

After HCT patients with TBDs may have delayed or impaired immune reconstitution compared with patients undergoing HCT for other disease indications [4]. Immune function assessment post-HCT is conducted at intervals determined by institutional HCT program guidelines, and include standard assessments listed above, B cell subsets to assess for recovery of immunoglobulin class switching, T cell receptor excision circle (TREC) testing to assess for recovery of thymic output, T cell spectrotyping to assess for diversity of reconstituted immunity, and mitogen stimulation testing to assess for T cell response potential [10]. Immunology consultation should be considered for post-HCT patients with impaired immune reconstitution that persists through one year post-HCT, including patients with absolute CD4 count less than 200/µL, absent TREC levels, absent B cell class switching, and low Ig levels at the one year visit. Re-immunization readiness after HCT should follow institutional BMT program guidelines and for patients with impaired immune reconstitution, initiation of re-immunization schedules should also involve discussion with a consulting immunologist.

Endocrinology and Bone Health Screening

Due to the high risk of endocrine disorders in patients with confirmed high-risk TBD, including growth/pubertal delay, hypogonadism, and bone health complications including pathologic fractures and avascular necrosis [11, 12], establishment of an annual screening plan with an endocrinologist is recommended by 10 years of age (see also Chapter 22, Endocrine and Skeletal Disorders). Patients with vertical growth deficiency detected on annual general pediatric screening, patients with symptoms or history concerning for compromised bone heath, and patients receiving androgen therapy for BMF should initiate formal endocrinology care earlier in the first decade of life once these issues arise.

Patients in the moderate risk TBD category likewise should initiate endocrinology care if concerning symptoms of growth failure or bone health compromise arise. Otherwise, these moderate risk patients may not require standing endocrinology follow-up care. In contrast, patients with certain high risk TBD subtypes, including Coats Plus and Hoyeraal-Hreidarsson syndrome [13, 14], that are known to be at high risk for pathologic fractures and other sequelae of compromised bone health should initiate endocrinology/bone health care at the time of diagnosis to enable early interventions that may prevent long-term bone sequelae. All patients who have undergone HCT for BM failure associated with TBD should have standing yearly endocrinology follow-up starting by the end of the first year post-transplant, given the likelihood that HCT may exacerbate pre-existing endocrine dysfunction.

In terms of specific endocrine screening and routine care by system:

- **Thyroid.** Individuals with TBD are at relatively low risk for baseline thyroid dysfunction compared to individuals with other inherited BM failure disorders. We recommend baseline serum free thyroxine (T4) and thyroid stimulating hormone (TSH) level assessment, but ongoing screening only for those with abnormalities detected on initial screening or if concerning symptoms arise. In contrast, all patients post-HCT for TBD should have annual serum free thyroxine

(T4) and thyroid stimulating hormone (TSH) levels assessed.

- **Bone health.** From the time of diagnosis, individuals should have annual screening of 25-OH Vitamin D levels assessed, with repletion recommended for levels below the normal range for age. Counseling to ensure adequate dietary intake of calcium is also important in maintaining bone health. Post-HCT patients require more frequent monitoring of Vitamin D levels and more aggressive repletion during the first year post-HCT per institutional guidelines. A baseline dual energy absorptiometry (DXA) scan should be performed between the ages of 12-14 years to assess for bone mineral density in high-risk TBD patients. Patients with normal scans and no history of osteopenia related complications should have follow-up DXA performed every 3 to 5 years, with frequency dependent on other disease manifestations. Post-HCT patients should have DXA evaluations beginning one year after transplant.

- **Growth/gonadal function.** Estimates suggest up to 20% of individuals with TBD exhibit short stature, though many of these patients do not have evidence of growth hormone deficiency [15]. Nevertheless, patients below the fifth percentile in height and in whom this height percentile is discordant with expected mid-parental height, should be evaluated by endocrinology with a hand x-ray for bone age and for GH axis function. Pediatric patients with growth deceleration post-HCT should also undergo GH axis evaluation. Patients with delayed pubertal onset or incomplete progression through puberty require endocrine consultation to assess LH, FSH, testosterone, and estrogen levels where appropriate. In contrast, patients with TBD treated with androgens for BM failure may exhibit accelerated linear growth, masculinization, and precocious puberty [16], and therefore need close monitoring by endocrinology (up to every 6 months) for these complications and their management.

Hepatic and Pulmonary Screening

While some hepatic and pulmonary complications of TBD may occur independently, growing evidence suggests a link between hepatic and pulmonary complications including fibrosis [17] as well as hepatopulmonary syndrome [18], in which the formation of intrapulmonary shunts is driven by the development of portal hypertension and portosystemic shunting. Therefore, screening for hepatic and pulmonary complications of TBD are linked and involve close coordination between hepatology and pulmonary physicians.

Liver screening assessments

Screening blood tests of liver function should be done at diagnosis and at least annually all patients with TBD, and should include transaminases, bilirubin, albumin, cholestasis markers such as gamma glutamyl transferase (GGT) and alkaline phosphatase, and prothrombin time. Starting at five years of age in patients with confirmed high risk TBD and/or starting one year post-HCT, liver ultrasound (US) should be performed annually to assess for architectural changes such as nodular regenerative hyperplasia and for tumors including hepatocellular carcinoma or angiosarcoma, both of which have been reported in patients with TBD [19, 20]. While not used universally, many centers are incorporating routine screening with ultrasound elastography (also known as a fibroscan) that assesses liver stiffness as a sign of fibrosis [21], in addition to traditional US imaging.

TBD patients receiving androgen therapy should undergo liver US as frequently as every 6 months due to increased risks of liver adenomas and peliosis. As it is non-invasive, a baseline screening liver US is still recommended by age 10 even in patients with suspected/moderate risk TBD. If normal, moderate risk patients should repeat this imaging every three to five years. Any patient who develops sustained LFT or liver US abnormalities should establish care with a hepatologist familiar with treating patients with TBDs.

Pulmonary screening assessments

Young children with a history of recurrent sinopulmonary infections and those requiring early HCT should establish care with a pulmonologist in early childhood. Initial evaluation in this subgroup of patients should include annual spirometry starting at age five years when developmentally appropriate, and a baseline high resolution, non-contrast computed tomography (CT) to assess for bronchiectasis, fibrosis, and areas of chronic infection (see also Chapter 14, Pulmonary Fibrosis). Repeat CT imaging is not performed routinely but can be considered if there is a significant decline in spirometry or development of new respiratory symptoms. Otherwise, patients with either confirmed/high risk or suspected/moderate risk TBD but who lack a prior history of pulmonary symptoms should establish pulmonology care and begin routine screening spirometry by age 10. Diffusing capacity (DLCO), a sensitive marker of pulmonary fibrosis, can also often be accurately assessed starting at age 10 depending on patient developmental status, and should be added to pulmonary function screening at this time. For patients without ongoing pulmonary symptoms and without prior HCT, PFT screening can be repeated every 2-3 years. For patients with TBD who are post-HCT, more frequent (at least annual) PFT evaluations are indicated, particularly for patients who received conditioning containing alkylating chemotherapy or total body irradiation (TBI) [4]. Patients undergoing HCT should have CT imaging pre-transplant, with post-transplant imaging indicated for new onset of pulmonary symptoms.

Hepatopulmonary syndrome

Hepatopulmonary syndrome (HPS), in which portosystemic shunting driven by endothelial dysfunction in the liver drives vascular malformations in the lung and subsequent hypoxia, has been increasingly recognized in the past decade as an underlying cause of progressive dyspnea occurring in the first 4 decades of life for patients with severe TBD [18, 22] (see also Chapter 18, Hepatic Complications). At this time, no routine screening in asymptomatic individuals has been adopted to assess for HPS, in part because doppler ultrasound of portal flow appears to be insensitive at

detecting early stages of HPS. Recent clinical experience suggests that contrast echocardiography (also known as a bubble study) may sensitively detect pulmonary AVM's in patients with TBD that result from HPS. At this time, contrast echocardiography is not part of routine screening for pediatric TBD but should be considered in any pediatric patient pre- or post-HCT who develops new-onset dyspnea without infectious cause and/or evidence of progressive non-cirrhotic portal hypertension.

Head, Neck, Dental, and Hearing Screening

All pediatric individuals with confirmed or suspected TBD should begin twice yearly screening evaluations with a dentist familiar with assessments for oral pre-cancer and cancerous lesions (see also Chapter 8, Dental and Oral Complications). Lesions persisting for more than two to three weeks should be biopsied. If the biopsy is negative for malignancy, lesions should be re-biopsied with any significant changes in size or appearance. In addition to mucosal lesions, patients with TBDs are at high risk for periodontal disease, dental decay, dental agenesis, and thin enamel [23]. Fluoride supplementation for young children, routine dental cleanings with precautions taken if low blood counts are coexistent, and meticulous daily dental hygiene are recommended for all patients with TBD.

Beginning at age 10, patients with confirmed/high risk TBD either pre- or post-HCT should establish care with an otolaryngologist to undergo nasolaryngoscopic evaluation of the nasal, oropharyngeal, and laryngeal mucosa for pre-malignant or malignant lesions. If the initial evaluation is normal, repeat screening can be performed every 2-3 years, whereas at least annual screening is recommended for patients in whom suspicious lesions are identified.

Hearing impairment is a feature of some, but not all TBD. Any toddler or older child with a TBD who exhibits significant speech delay or fails an independent hearing assessment should be referred for formal audiologic assessment.

Gastrointestinal (GI) and Nutrition Screening

All pediatric individuals with TBD should have weight and nutrition assessments at a minimum of every 6 months performed by their general pediatrician and/or medical home provider. Weight loss, inadequate weight gain, or other metrics consistent with failure to thrive are indications for formal consultation with nutritionist. Even after adequate nutrition has been restored and supplementation is discontinued, ongoing annual nutritionist consultation is recommended to ensure adequate number of calories and a balance of nutritional sources continues. After HCT, patients are at particularly high risk for malnutrition due to chemotherapy effects on the absorptive capacity of the GI tract, infections and medications that alter gut flora, and possible complications from graft versus host disease. Thus, weekly to monthly nutrition consultations are often required during the first year post-HCT.

Patients with TBD are also at high risk for developing esophageal stenosis and esophageal webs [24] (see also Chapter 17, Gastrointestinal Disease). At twice yearly visits, all patients should be screened for onset of difficulty swallowing, regurgitation, and new solid food avoidance. A positive screen would indicate need for referral to a gastroenterologist with experience in performing and interpreting esophagrams and esophagoscopy with dilatation in patients with TBD. Patients who have previously developed esophageal stenosis often require yearly evaluations with an interventional gastroenterologist for consideration of repeat esophagoscopy and dilation procedures.

Patients with TBDs may also develop enteropathy and enterocolitis [24]. Patients with high or moderate risk TBD who have chronic abdominal pain, diarrhea, and specific food intolerance related to these symptoms should be referred to establish routine care with a gastroenterologist. Hematochezia (bloody stools) in patients with TBD may be a sign of enterocolitis, GI telangiectasias (particularly in Coats Plus), or lower GI tract malignancy and warrants urgent evaluation by a gastroenterologist [25].

Esophageal, stomach, colon and rectal cancer have all been described in patients with TBD [26]. In patients with confirmed/high risk TBD not undergoing routine upper and lower endoscopic screening for the above complications, routine esophagogastroduodenoscopy and sigmoidoscopy/colonoscopy to screen for malignant or pre-malignant lesions should be initiated by age 18. If initial screening is normal, endoscopies should be repeated every three to five years. In addition, fecal occult blood (FOB) screening by card-based testing should be started around age 12. For patients with suspected/moderate TBD, FOB and endoscopic screening should be performed for any patients with a history of chronic GI symptoms. Finally, patients who have undergone HCT should have FOB testing initiated with one year after HCT and endoscopic screening initiated within three to five years regardless of age, particularly if they have had a history of GI complications associated with HCT including GVHD.

Dermatologic Screening

For all pediatric individuals with TBD, routine screening for skin squamous cell carcinoma (SCC) should be performed with annual visits to a dermatologist starting at age five or earlier if concerning lesions are present, given the early known occurrence of SCC in patients with TBD [6] (see also Chapter 6, Dermatologic Manifestations). This yearly dermatologic follow-up may also be helpful for management of the many other dermatologic complications that can occur with TBD, including hyperkeratosis (thickening) of palms/soles, poor hair growth/alopecia, and hyperhidrosis (excessive sweating) [27].

Starting from the time of diagnosis, routine daily skin care is critical for optimal dermatologic outcomes. This care includes strict adherence to sun protection strategies including liberal use of sunscreen, use of hats and clothing to avoid sun exposure, and limiting peak UV ray exposure during the hours of 10 am to 4 pm. Daily moisturizer use, avoidance of harsh soaps/cleansers, and excellent oral hydration intake are additional critical elements to successful routine dermatologic care.

Ophthalmologic Screening

Patients with specific TBD including Revesz syndrome, Hoyeraal Hreidarsson syndrome, and Coats Plus are additionally at high risk for retinal vascular changes including exudative retinopathy and neovascularization [28]. These patients should have a formal retinal vascular evaluation upon diagnosis, to prevent vision loss (see also Chapter 7, Ophthalmologic Complications). Routine annual eye exams for all patients with TBD should begin by age five years to screen for the many ophthalmic complications common in TBD, including nasolacrimal duct obstruction/collapse leading to excessive tearing, ectropion and loss of eyelashes, corneal scarring and conjunctivitis [29]. After HCT patients are at risk of dry eyes and cataracts due to GVHD and conditioning agent side effects, respectively.

Neurologic Screening

Developmental assessments and neurologic exams should be performed on all pediatric individuals with TBD (see also Chapter 24, Neuropsychiatric Complications). Many patients with TBD do not manifest overt neurologic symptoms or verbal/cognitive developmental delay. Thus, onset of developmental delay or any symptoms of a neurologic disorder including tremor, focal weakness/paresthesia or seizures should warrant neurology consultation. A screening brain MRI is recommended for any patient with developmental delay or neurologic symptoms. Screening brain imaging by MRI and/or CT imaging is also recommended as an initial screen for all patients with TBDs genetic variants highly associated with neurologic complications. These variants include Hoyeraal Hreidarsson syndrome which is associated with cerebellar hypoplasia, and Coats Plus and Revesz syndromes that are associated with intracerebral calcifications [30]. Patients with these specific syndromes are recommended to have routine annual care with a neurologist to assess and manage onset or progression of neurologic symptoms.

Behavioral and Mental Health Screening

Patients with TBDs are at increased risk for early childhood onset of attention deficit and hyperactivity disorder, pervasive developmental disorder (eg. Autism), and learning disorders [30, 31] (see also Chapter 24, Neuropsychiatric Complications). Pediatricians and medical home providers should assess for these symptoms at twice yearly visits in early childhood. Positive screens for any of the above conditions should result in referral to community-based resources such as early intervention, school-based resources for the development of an individualized education program (IEP), and/or private behavioral health resources for evaluation and management.

Due to the complex medical care and challenging prognoses associated with many of the disease manifestations of TBDs, patients are at high risk for development of mood, anxiety, and adjustment disorders related to coping with a chronic medical illness. We recommend older pediatric and adolescent patients establish care with a mental health specialist familiar with TBD and associated complications, preferably by age 10-12 years. Routine counseling, at least annually, with this specialist that includes discussion of individual stressors and general coping strategies, is an essential part of comprehensive care for patients with TBD.

Table 1: Screening guidelines for pediatric patients with confirmed High-risk Telomere Biology Disorders (TBD).

Specialty/ Type of Screening	Timing of Initial Screen*	Frequency of Follow-up Screening (if initial screen normal)+
Hematology		
CBC monitoring	At diagnosis	Every 6 months
Bone marrow monitoring	At diagnosis	Yearly starting at 10 years
Immunology		
Immune function assessment	At diagnosis	Repeat with change in infection frequency or development of BM failure
Endocrinology		
MH growth and bone health assessment	At diagnosis	Yearly
Endocrinology consultation	Age 10[&]	Yearly
DXA scan	Age 12-14	Every 3 to 5 years
Hepatology		
Liver function tests	At diagnosis	Yearly
Liver ultrasound	Age 5	Yearly[#]
Pulmonary		
Spirometry/DLCO	Age 10[$]	Every 2-3 years
High Resolution CT	At diagnosis, if history of symptoms	Based on symptoms
ENT/Oral Cavity		
Dental assessments	Age 3	Every 6 months
Nasolaryngoscopy	Age 10	Every 2-3 years

Audiology	Only if history of symptoms	Based on symptoms
GI/Nutrition		
MH nutrition assessment	At diagnosis	Every 6 months
Nutritionist consultation	At onset of FTT	Yearly
Upper/lower endoscopy	At symptom onset or age 18	Every 3-5 years for routine screening. Yearly if symptoms.
Dermatology		
Dermatologist screening	Age 5	Yearly
Ophthalmology		
Routine eye exam	Age 5	Yearly
Retinal exam for specific TBD[%]	At diagnosis	Yearly
Neurologic		
Brain MRI for specific TBD[%] or neurologic abnormalities	At diagnosis	Based on symptoms
Behavioral/Mental Health		
ADHD, PDD, learning disorders	At diagnosis	Depends on symptoms
Mental health assessment	Age 10	Yearly

Abbreviations: BM, bone marrow; MH, medical home; hx, history; GI, gastrointestinal; FTT failure to thrive; ADHD, attention deficit/hyperactivity disorder; PDD, pervasive developmental disorder

*Recommendations in this table refer to patients who have not undergone hematopoietic cell transplant (HCT) and have confirmed severe TBD. Recommendations for post-HCT monitoring and for patients with suspected/moderate TBD are included in the chapter text.

+More frequent monitoring is generally recommended for patients with abnormal initial screening, as detailed in the chapter text

&Patients with growth delay, compromised bone health, or receiving androgen therapy need earlier initiation of endocrinology care

#Patients receiving androgen therapy need liver ultrasound screening every 6 months

$Patients with history of sinopulmonary infections or lung disease in early childhood should begin spirometry at age 5. Age of initial PFT's is also dependent on developmental ability.

%TBD at high risk for retinal and neurologic abnormalities include Revesz, Hoyeraal-Hriedarsson, and Coats Plus syndromes

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Chapter 26

Transitioning from Pediatric to Adult Medical Care

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"It takes courage to grow up and become who you really are." — E.E. Cummings

"To exist is to change, to change is to mature, to mature is to go on creating oneself endlessly." — Henri Bergson

Introduction

Children and adolescents with chronic medical conditions face an uphill battle as they navigate the challenges of becoming an adult. Chronic medical conditions lead to reduced school participation that can impair school performance and ultimately limit employment attainment. Decreased community participation due to medical care needs and concerns about body image may lead to decreased

practical knowledge regarding independent living and increased rates of depression, anxiety, and social/legal problems [1, 2].

Individuals with telomere biology disorders (TBD) come to the challenge of adulthood from differing crossroads depending on the history of their disease. For some patients with TBD diagnosed in early childhood, the major challenge may be navigating a transition from pediatric to adult medical providers across a wide swath of specialties while trying to maximize educational attainment or entering the workforce. For others, the diagnosis of a TBD may not be made until late adolescence, forcing teenagers to cope with a new diagnosis that has the potential to greatly disrupt preset plans for college and beyond. Finally, other individuals with TBD may not yet be aware of their diagnosis but may be experiencing fear and stress of symptoms that have yet to be explained.

While the burdens of having a chronic medical condition such as TBD are immense, there are some benefits when contemplating a successful transition to adulthood. Taking ownership of complex medical care leads to maturity that may serve patients well in college, initial employment, and beyond. In addition, resilience learned through years of overcoming medical challenges can make navigating the highs and lows of adjusting to the workforce and other aspects of the adult world easier. The purpose of this chapter is to discuss how TBD individuals, families, and their providers can facilitate the process of transition from pediatric to adult medical care to make this potentially stressful transition go smoothly during what is already a challenging time for young adults and their families.

What is Pediatric to Adult Transition?

The Society for Adolescent Medicine recognized the need for formalized pediatric to adult transition for patients with chronic conditions in the early 1990s, stating that these patients would benefit from "the purposeful, planned movement of youth with special health care needs from child-centered to adult-oriented care" [3]. Nearly three decades later, despite extensive research and clinical initiatives, only 17% of youth with special health care needs meet benchmarks for having sufficient transition planning [4]. For TBD patients, the pediatric to adult transition is made particularly challenging due to the rarity of TBD and the lack of familiarity of its management by many pediatric and adult providers. Additionally, diverse disease presentations involving multiple organ systems require multispecialty care coordination. Patients may have difficulties maintaining adequate healthcare insurance coverage and identifying a suitable adult "medical home" to both facilitate the transition to adult care and provide long-term care continuity once the transition to adult care has been completed.

You Are Not Alone

The challenges associated with the transition from pediatric to adult health care are not unique to individuals with TBD. Approximately 10 million children (~20%) in the United States have chronic diseases that qualify as special health care needs, and at least 500,000 individuals with these childhood-acquired conditions requiring transition planning each year [5]. Recognizing the need for a systematic approach to optimize care for this young adult population, the American Academy of Pediatrics, the American Academy of Family Physicians, and the American College of Physicians-American Society of Internal Medicine issued a consensus statement in 2002 that included several specific recommendations for transition [6]. These recommendations have recently been updated [7], focusing on six core elements of preparation for both the pediatric and adult practices involved in patient care transition (Table 1).

Table 1. Six core elements of pediatric to adult transition.

Responsibilities	Pediatric Practice	Adult Practice	Tips for Parents/ Families to Ensure Successful Transition
Transition care policies/ procedures	● End date of care provision ● Services/resources provided	● Age-based care start date ● Patient acceptance policy	Ask your team about their policies
Tracking and monitoring	● Youth/family preparation	● Review of relevant records	Ask how tracking is performed in your center
Readiness/ orientation	● Formal readiness assessments	● Welcome and FAQ documents to orient young adults to practice	Start discussing readiness approach by age 14
Planning and integration	● Develop individualized transition plan ● Medical summary ● Communication	● Communication with pediatric clinician ● Receipt of transition package	Basic plan should be in place by age 18 even if transition occurs several years later
Transfer	● Final visit ● Define pediatric responsibilities to be retained during transition	● Initial visit ● Self-management skills assessment	Ensure that the first adult practice appointment is scheduled by the date of last pediatric appointment
Transition completion	● Seek feedback on transition ● Confirmation of ongoing adult practice appointments	● Ongoing care ● Self care skill building ● Communication with prior providers when necessary	Ensure that the pediatric and adult practices are communicating over at least the first 6-12 months of the transition

Pediatric and Adult Care in TBD:
Different Styles but Same Need for Medical Home

Pediatric and adult care models are broadly different (Table 2). Young adults transitioning to the adult care model need to be aware of these differences to avoid lapses in quality of care. The task of establishing a "medical home" in the adult care system is an additional aspect that can be particularly challenging for TBD individuals who frequently have multispecialty care needs. The medical home model is a way of delivering health care that is coordinated, patient and family centered, and culturally appropriate [7]. In this model, the medical home is the provider who serves as the hub of the medical wheel, keeping track of the current status and screening needs across the spectrum of a patient's health care needs. Proven benefits of utilizing a medical home care model for patients with complex medical conditions like TBD include:

- Easier access to services
- Consistent and coordinated care
- More efficient and effective use of resources
- Better support to individuals and families
- Improved health, developmental, educational, vocational, psychosocial, and functional outcomes

The adoption of this model is sometimes more challenging for rare complex diseases, such as TBD, because the medical home provider needs to have a good understanding of all aspects of TBD. For children with TBD, subspecialists at large academic centers such as pediatric hematologists, immunologists, geneticists or stem cell transplant physicians frequently provide this medical home. In the United States, due to special provisions by Medicaid and private insurance policies for pediatric patients, pediatric academic practices can overcome insurance barriers to seeing patients who may be "out of network" more readily than can many adult practices. Tertiary care pediatric

practices are also frequently equipped to handle patient complexity and have established connections with multiple subspecialists required for TBD patient care.

Table 2. A comparison of pediatric vs. adult care.

Pediatric Care	Adult Care
Parents and medical providers oversee care and monitor symptoms	Care is self-directed and self-monitored by patients
Parents make treatment decisions and may shield patients from details regarding prognosis	Patients have final say in treatment decisions and must be able to handle discussing prognosis
Parents schedule appointments and find new providers as needed	Patients must schedule and keep appointments and find new providers on their own
Late appointments and last-minute rescheduling often tolerated	Being late or rescheduling appointments at the last minute may result in significant delays in care
Transportation provided by parents	Patient must provide own transportation
Social work and behavioral health services are integrated into pediatric subspecialty clinics	Patient must seek outside support services for financial and emotional issues
Parents are responsible for maintaining insurance coverage and healthcare payments	Patient is responsible for maintaining insurance coverage and healthcare payments
Parents manage home medications and home care treatments	Patient must obtain prescriptions and refills and manage home care needs

In the United States, at the time of transition to adult care, some TBD patients may encounter restrictions on adult subspecialty practices that are "in-network" with their insurance. While there are notable exceptions, adult subspecialty practices that are "in-network" may not be equipped with the same resources and connections to efficiently provide this same medical home after patients have transitioned to adult care. Another important distinction is that adult hematology and immunology physicians see fewer patients with TBD and are more familiar with later onset TBD presentations. This is in part because many patients with TBD have undergone

hematopoietic cell transplantation (HCT) by the time of adult care transition. Consequently, adult hematology or immunology practices are not as central to the care of patients who have already undergone stem cell transplant, and are less familiar with complications seen in patients with more severe, pediatric-onset disease.

Options for maintaining a medical home for patients with TBD transitioning to adult care models thus include the following:

- Identify a regional expert in the care of TBD who practices in the adult care space that is willing to serve as a medical home. While historically such providers were rare, there are fortunately increasing numbers of internal medicine-trained subspecialists who are developing familiarity and expertise in caring for patients with TBD. At the time of transition, your pediatric provider can help provide a list of potential adult care providers in your area. Patients and families can work proactively with a social worker or insurance specialist to identify adult tertiary care practices or consider alternative insurance options to ensure access to a tertiary care center with TBD expertise. However, unfortunately, in the United States, "in-network" insurance restrictions may still create a barrier to accessing a specific regional center.
- Establish care in a dedicated primary care physician (PCP) office with providers trained in internal or family medicine who have experience in managing patients with chronic medical conditions. While this model can be quite successful, it does require establishing communication between this PCP and regional experts in TBD management to ensure an adequate level of TBD expertise.
- Maintain a medical home provider based in a pediatric practice, while transitioning subspecialty care to adult care providers. This option provides optimal continuity by including a provider with first-hand knowledge of a patient's medical history in their adult care. However, this option requires that the pediatric provider's hospital system, subspecialty practice, and the patient's insurance plan allow ongoing medical care of adult patients within a pediatric practice.

Timing of Transition

At age 18, a person has the right to vote, make medical decisions, provide written informed consent to research studies or treatments, control who has access to their medical information, designate a health care power of attorney/health care agent, and create an advanced directive/living will. Whereas this legal transition from childhood to adulthood is abrupt, the real-world transition to adulthood is a gradual process that differs widely among individuals. Moreover, the transition from pediatric- to adult-oriented health care coincides with emerging adulthood in other life domains such as education, employment, social relationships, and independent living.

Ideally, the knowledge and skills needed for transition are learned over time and tailored to the developmental stage of the individual (Table 3). Young children are primarily recipients of care, with management provided by parents and medical providers. Grade School-age children should be provided with developmentally appropriate information and can often begin to participate in aspects of their care. As patients mature through adolescence, parents and providers should make efforts to engage adolescents in a shared management model, thereby beginning the transition process by having teens play an active participatory role in their care management [8]. By the end of the transition process, patients should have the skills to be the primary supervisors of their care, with parents and providers primarily serving as resources and consultants.

Table 3. Role transition in care management from early childhood to adulthood.
Adapted from Kieckhefer & Trahms, *Pediatric Nursing*, 2000.

Age	Provider	Parent/Family	Patient
Early Childhood	Major responsibility	Provides care	Receives care
Grade School Age	Support to family and patient	Manager	Participates
Secondary School Age	Consultant	Supervisor	Manager
Adulthood	Resource	Consultant	Supervisor

Determining Readiness: One Size Does Not Fit All

A number of factors influence transition readiness for individuals with chronic health care needs. In a 2013 study of adolescent and young adult survivors of childhood cancer, a stakeholder validation analysis identified several factors critical to successful transition [9], of which some pre-existing factors can be challenging to change, while others can be modified through a comprehensive transition program (Table 4). For example, while sociodemographic factors, health care access, and global cognitive ability are often static factors, at least in the short term, efforts to promote medical literacy, self-sufficiency, and even anger management/maturity skills through training can greatly facilitate the success of pediatric to adult transition.

Table 4. Patient-specific factors influencing transition timing. Adapted from Schwartz et al, 2013.

Pre Existing Factors	Modifiable Factors
- Social and demographic factors - Health care access/insurance - Medical status - Cognition	- Knowledge - Self-sufficiency - Expectations - Maturity - Motivation - Communication - Emotions

In practice, for most youth, transition to adult care takes place around the age of 22. However, because the pediatric−adult transition process is influenced by a variety of personal, condition-specific, and sociocultural factors, there is no one set of recommendations and milestones applicable to all individuals, particularly in a heterogeneous disease such as TBD where the care needs are widely variable. There are, however, clues and milestones that suggest a young adult is ready for transition to adult care. These include:

- Lack of comfort in addressing important areas of health with a pediatric provider
- Feeling out of place in the overall environment of a pediatric clinic
- The preferred family support at clinic visits is a boyfriend, girlfriend, or spouse as opposed to a parent, or if a patient is themselves a parent.
- Subspecialists involved in the care team begin to require transition per hospital policies
- Completion of formal education

Disease-Specific Factors Influencing the Timing of Transition in TBD

Patient-specific, pre-existing factors heavily influence the timing and nature of the transition to adult care for patients with TBD. For those with severe forms of TBD in whom the diagnosis has been made in early childhood, the focus of pediatric-adult transition is often transitioning multiple previously established subspecialty services to adult care. For these patients, this transition may be occurring amid an overall adjustment to the myriad of challenges of young adulthood while they continue to have multiple long-standing medical limitations. In other cases, TBD may be diagnosed in late adolescence and may present with bone marrow failure or perhaps liver or hepatopulmonary concerns. These patients may need to decide whether to establish new subspecialty care in pediatric versus adult centers at a time where they are still learning about their diagnosis and what medical services they may need.

Because TBD is a rare condition, there are significant geographical differences in access to disease-specific expertise. Tertiary care options may also be quite limited for patients living in some regions. This limited access may result in patients being justifiably reluctant to leave local pediatric providers with whom they have a strong, well-established relationship. A related problem that sometimes limits access to expert care is that private- and state-sponsored health insurance programs for children are often flexible in allowing children with chronic medical needs to seek out medical expertise, even if this means crossing state or provincial boundaries. In contrast, health insurance programs for adult patients may impose restrictions in accessing the same degree of expertise in adult care centers, particularly if expert providers are not a part of the treatment network.

Another major factor in deciding the optimal time to transition to adult care is the set of organ-specific disease manifestations in individual TBD individuals (Figure 1) and the availability of adult subspecialty providers in their local area that can address these

specific complications (Figure 1). For example, expertise in severe immune deficiency, developmental concerns, and even enterocolitis associated with TBD that often occur in early childhood are most readily found in pediatric health systems. In contrast, complications that are seen more frequently in adults with TBD, such as pulmonary fibrosis, head and neck cancer, and end-stage liver cirrhosis, are more frequently cared for by adult medicine practices. Complications that come to the forefront in adolescence and young adulthood, such as bone marrow failure (BMF), development of myelodysplastic syndrome (MDS) or leukemia, worsening gastrointestinal hemorrhage seen in some TBD patients [10], and the increasingly recognized hepatopulmonary syndrome [11] often present the most challenges when considering transition. While expertise in BMF/MDS diagnostics and treatment with HCT typically exist in both pediatric and adult tertiary care practices, patients with GI hemorrhage or hepatopulmonary syndrome may be reluctant to transition to adult care while the acuity of these complications is increasing, despite the fact that adult liver transplant and interventional gastroenterology practices may have greater expertise in managing these complications. Thus, for young adult individuals who are struggling with these complications, it is critical to ensure a comprehensive transition plan focused on maximizing communication between pediatric and adult care teams across subspecialties.

Figure 1. Example of how medical expertise for the distinct age-based disease manifestations of TBD may be divided between pediatric and adult practitioners.
Note: The depicted spectrum may not accurately reflect expertise at all centers, and age of onset of disease manifestations may differ by patient.
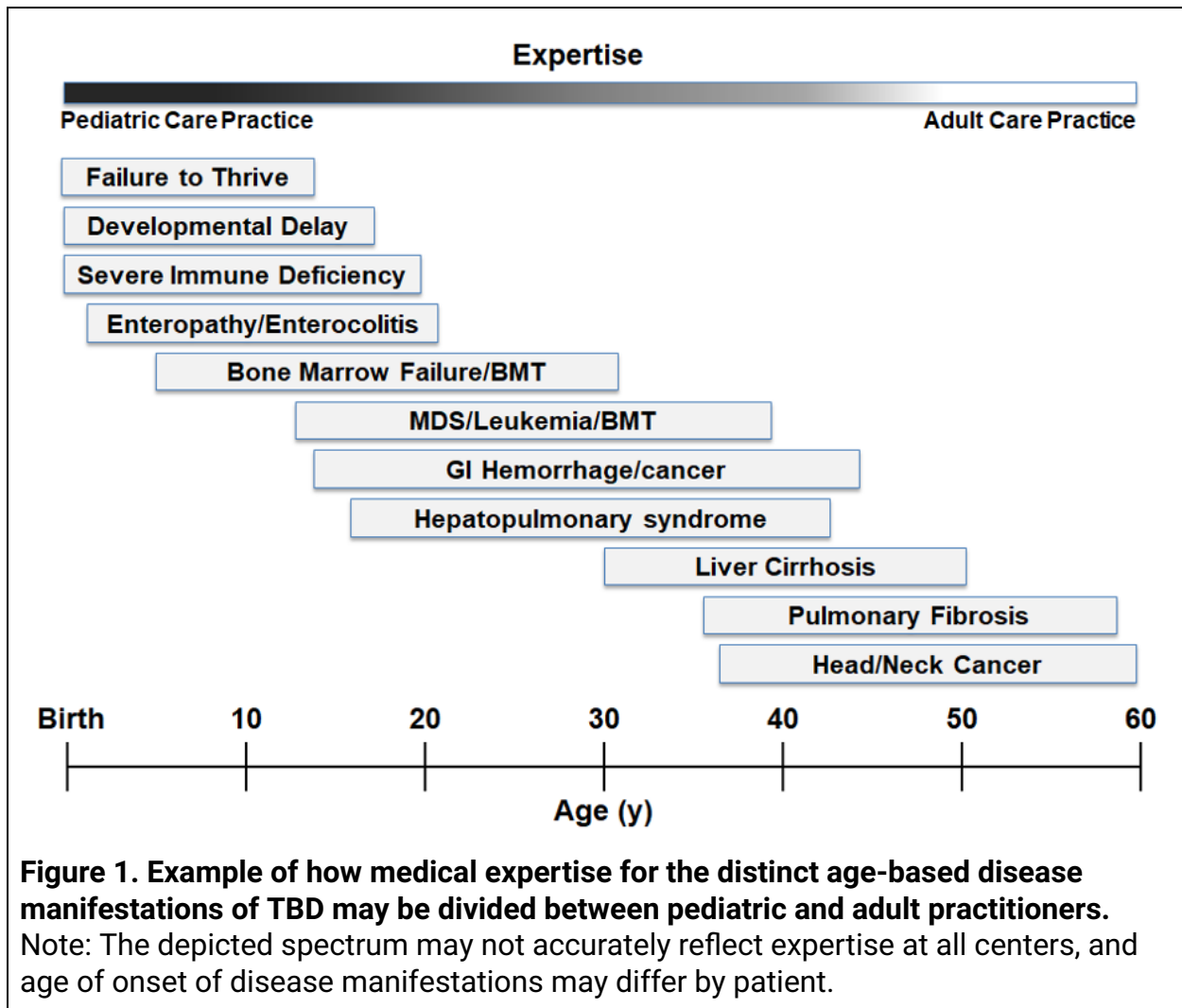
Unfortunately, many patients with TBD also experience neurocognitive or neuropsychiatric deficits. In severe forms of TBD such as Hoyeraal-Hreidarsson and Revesz syndromes, the severity of neuropsychiatric deficits may make the transition to independent medical management infeasible. Nonetheless, many of these individuals will develop complications that are best managed using expertise from adult providers. These patients particularly benefit from formal identification of a medical home, whether from a pediatric provider or one specializing in caring for adults with disabilities, as long as this medical home can involve all necessary subspecialists to create an individualized, cohesive network of care.

Persons with neurocognitive disabilities may have the capacity to designate a healthcare or medical power of attorney, even if they lack the capacity to make more complicated medical decisions for themselves [12]. In other cases, state or other government oversight institutions may have laws or procedures to automatically designate a family member to act as the surrogate decision-maker for individuals who lack the capacity to make medical decisions and are unable to designate a medical power of attorney. Therefore, when significant neurocognitive concerns exist, transition planning should also include assistance with the process of pursuing legal guardianship for families who wish to do so.

General Tips for Focusing on Modifiable Factors to Improve Transition Outcomes

Despite the challenges encountered during the transition process, families and providers who focus on factors that are most easily modifiable (Table 4) can overcome many of the potential obstacles and ensure optimal care through this critical period and beyond. In particular, teens must gain the necessary skills and the knowledge necessary to maintain high-quality health care.

As a basic guideline, between the ages of 11 and 13, most patients should be developmentally able to understand their medical condition and their basic health care needs. This includes being able to:

- Briefly explain their condition to others
- State which doctors they see and for what reason
- State what medicines they take and for what reason
- Take their medications without reminders
- Know their allergies

Patients in this 11-13 year-old age range, with the help of techniques such as a 3-sentence health summary (Table 5) should be able to easily discuss health information with their regular providers, as well as in emergency situations.

By 14 to 16 years of age, developmentally able patients should be directing most of their communications with health care providers. They should also have the opportunity to discuss aspects of their care privately with providers. They should fully understand emergency health care plans and be engaged in accessing community resources to help them navigate school/work challenges posed by their medical condition. In addition, they should begin to understand the processes of health insurance and making appointments.

By late adolescence and early young adulthood (17-22 years of age), patients should be able to schedule their own appointments and arrange necessary transportation. They should have full knowledge of their health insurance coverage and have a sense of how they will maintain coverage in the future, based on their career plans and goals. They should also play the primary role in maintaining health records by using a "care binder" notebook or another system for organizing health information. They should be able to update and maintain access to written health summaries and emergency care cards. Resources that can help adolescents and young adults acquire these skill sets are listed in Appendix 1.

Table 5. Patient-specific factors influencing transition timing. Adapted from Schwartz et al, *JAMA Pediatr* 2013.

3-Sentence Health Summary

Method:
    Sentence 1: State your age, diagnosis, and most important points about your medical history.
    Sentence 2: State any treatment you are currently receiving.
    Sentence 3: State what your concerns or symptoms are now.
 	
Example:
1. I am 16 and have a Telomere Biology Disorder called dyskeratosis congenita. I have bone marrow failure and problems with my immune system, and I have had several severe lung and skin infections.
2. I receive red blood cell and platelet transfusions once in a while when my counts are low and have required admission to the hospital before when I have had an infection.
3. I am here today because I have a fever and a cough, and I just don't feel well.

How Can Parents Help?

While turning over autonomy for healthcare to their adolescent and young adult children can be understandably anxiety-provoking, empowering their child with the skills and confidence to manage their own care are the most critical contributions parents can make to ensuring continued high-level care. A few general tips for how parents can facilitate their child's transition are:

- Start early
- Teach developmentally appropriate information about your child's condition
- Encourage your teen to assume responsibilities
- Provide coaching opportunities and practice independence
- Help your teen understand the future health implications of their condition
- Discuss career and educational goals and their impact on health insurance
- Address decision-making and guardianship issues when necessary

Transition as a Challenge That Must Be Met

Becoming a successful young adult is challenging, even for adolescents who do not have chronic medical conditions such as TBD. Transitioning medical care from pediatric into adult systems is unmistakably an added burden for young adults with TBD during this already difficult developmental period. However, youth who experience a successful, systematic transition have better health outcomes and report improved rates of fulfillment, achievement, and higher self-esteem compared to those who do not attain a smooth transition. Pediatric and adult care providers, along with parents and other family members have an obligation to provide these individuals not only with exceptional medical care, but also the knowledge and skills necessary to ensure an optimal transition experience.

"Every new beginning comes from some other beginning's end." — Seneca the Younger

Appendix: Tools and Resources

Websites Focused on Pediatric to Adult Transition and Medical Home Resources

- National Health Care Transition Center: http://www.gottransition.org
- American Academy of Pediatrics:
  https://medicalhomeinfo.aap.org/Pages/default.aspx
- Florida Health and Transition Services: http://www.floridahats.org
- New York State Institute for Health Transition Training for Youth with Developmental Disabilities: https://healthytransitionsny.org
- Children's Hospital of Philadelphia Transition to Adulthood:
  http://www.chop.edu/centers-programs/transition-adulthood-program
  https://policylab.chop.edu/sites/default/files/pdf/publications/Transitions_Of_Care.pdf

Building a Health Care Summary and Maintaining Medical Records

- 5-page health summary:
  - https://www.acponline.org/system/files/documents/clinical_information/high_value_care/clinician_resources/pediatric_adult_care_transitions/gen_hema/general_hematology_clinical_summary.pdf
  - https://www.hematology.org/education/clinicians/clinical-priorities/pediatric-to-adult-care-transition
- Care binder:

  https://www.medicalhomeportal.org/living-with-child/after-a-diagnosis-or-problem-is-identified/caring-for-children-with-special-health-care-needs/managing-and-coordinating-care

Emergency Plans/Cards

- My Health Passport: http://www.sickkids.ca/MyHealthPassport
- Emergency Information Card:

  https://www.redcross.org/content/dam/redcross/National/m4240194_ECCard.pdf

Tips for College

- https://www.familyeducation.com/a-parent-guide-to-managing-chronic-illness-in-college

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Chapter 27

Routine Healthcare for Adults with Telomere Biology Disorders

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Introduction

Health care maintenance for people with telomere biology disorders (TBDs) can be complex due to the multisystem nature of these illnesses. This chapter summarizes current guidance based on expert opinion and experience of the authors.

Hematology

Hematologic abnormalities are very common in individuals with TBDs, from isolated (asymptomatic) macrocytosis or thrombocytopenia to severe aplastic anemia and myeloid neoplasia (see Chapter 10, Medical Management of Bone Marrow Failure in Telomere Biology Disorders). When an adult individuals is diagnosed with a TBD, hematopoiesis should be assessed to identify marrow failure and/or malignant transformation:

- Complete blood count (CBC) with differential and reticulocyte count
- Bone marrow aspiration
- Bone marrow biopsy
- Flow cytometry of bone marrow mononuclear cells (BMMCs)
- Conventional and molecular cytogenetics
- When available, Next Generation Sequencing (NGS) myeloid panel to assess somatic variants/clones

If evaluation provides evidence of aplastic anemia or myeloid malignancy (excess blasts, abnormal cytogenetics, marrow clonality), the patient should be referred to a hematologist/specialist to consider specific therapies (androgen, hematopoietic cell transplant, etc.).

If the CBC is normal or demonstrates mild cytopenias/abnormalities (e.g., macrocytosis, platelets >100/μL), blood and marrow may be monitored annually. However, if cell counts are falling, blood and marrow should be monitored more frequently.

Immunology

Immune dysregulation is often seen in patients with inherited or acquired marrow failure, including those with telomere-biology disorders. Thus, adult individuals

diagnosed with telomere diseases should be evaluated at presentation for cellular and humoral immunologic parameters:

- Flow cytometry for peripheral blood leukocytes including lymphocyte subsets (T CD4+, T CD8+, B cells, NK cells);
- Serum immunoglobulin levels (total and fractions, IgG, IgA, IgM);
- Depending on the patient's history, determine serum levels of tetanus/diphtheria/poliomyelitis/pneumococcal antibodies.

When immunodeficiency is detected, the patient should be referred to an immunologist.

Dermatology

Because of the increased risk of skin cancer, prevention strategies are highly recommended for individuals with TBDs/Dyskeratosis congenita (DC) (see also Chapter 6, Dermatologic Manifestations). Recommended strategies include:

- Regular use of sunscreen or sunblock when outdoors, and use of a daily moisturizing lotion with sunblock
- Wear hats and sun-protective clothing when outdoors to prevent excessive sun exposure
- Limit outdoor time during hours of peak sun exposure (between 10am and 4pm)
- Be mindful of reflected sun from water and snow when engaging in outdoor activities
- Avoid tanning beds
- Perform regular skin self-examinations to look for new or changing skin growths

In addition, an annual full body skin examination by a dermatologist is recommended.

Ophthalmology

Ophthalmic abnormalities are common and variable in children with DC/TBDs [1] and more severe forms, including Revesz syndrome and Coats plus syndrome [2], but are less frequent among adults with TBDs (see also Chapter 7, Ophthalmologic Complications) [3]. When the diagnosis of a telomere-biology disorder is confirmed in an adult individual, careful ophthalmic examination should be performed covering anterior and posterior segments and adnexa.

In addition to visual acuity, examination should address corneal changes, nasolacrimal duct obstruction, trichiasis in the adnexa; corneal lesions in the anterior segment; and cataracts, and retinal changes (vasculopathy, exudate, telangiectasias) in the posterior segment [2]. Unusual cases of exudative retinopathy may present late at adulthood [4]. If changes are observed, careful evaluation and follow-up with an ophthalmologist is warranted.

Dental

Individuals with TBDs are at increased risk of oral head and neck squamous cell carcinoma (see also Chapter 8, Dental and Oral Complications and Chapter 9, Solid Tumors). Adults with TBDs are advised to establish routine care and annual visits with an otolaryngologist (ear, nose, and throat physician) to screen for oral cancers. Monthly oral self-examination is recommended and can be done by the patient or a family member after ENT education. Patients should have a low threshold for evaluation if they note oral changes lasting more than two or three weeks.

Some patients with TBDs have dental anomalies, including short roots and widened pulp chambers. General hygiene recommendations include brushing teeth two to three times a day with fluoridated toothpaste and flossing once a day at a minimum to help prevent tooth decay. Some dentists recommend using prescription strength fluoride toothpaste or antibacterial mouth rinse to aid in reducing oral disease. Biannual dental

checkups and cleanings are recommended to monitor for the presence of oral pathology and prevent the development of significant dental decay and gum disease. Precautions during routine dental treatment may be necessary in the presence of low platelet counts and white blood cell levels.

Pulmonary

Large, prospective studies of pulmonary screening for asymptomatic individuals with TBDs have not yet been conducted (see also Chapter 14, Pulmonary Fibrosis). Figure 1 provides an algorithm for consideration of screening and routine follow up (as carried out at Mayo Clinic). Please note that these recommendations vary from institution to institution as there is lack of prospective evidence on which to base these screening recommendations. Several pulmonary screening methodologies are available including high-resolution chest computed tomography (HRCT), chest x-ray, and pulmonary function tests (PFTs). The two principal decision points in asymptomatic patients are long-term safety, primarily related to radiation exposure (measured in millisieverts [mSV]), versus sensitivity and specificity of the testing modality to identify interstitial lung disease (ILD). To put it into perspective, the average annual exposure to radiation in the environment is 3 mSV, compared to 7-15 mSV with chest CT scans and 0.02-0.1 mSV with chest X-rays [5] (see also Chapter 23, Radiation and Telomere Biology Disorders).
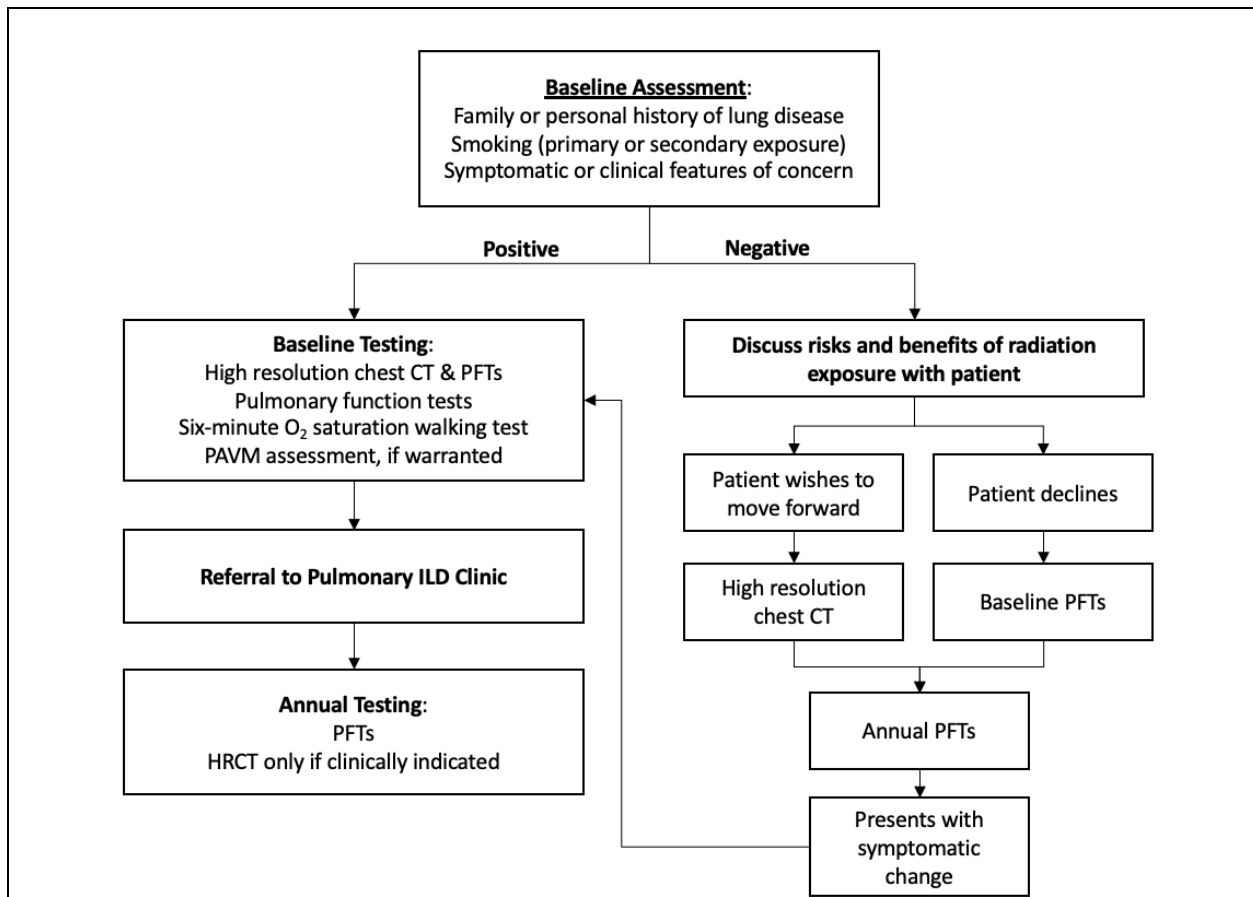
Figure 1: Pulmonary function assessment in telomere biology disorders. These recommendations should be tailored for each specific patient in consultation with their medical team.*

*These are the recommendations of the Mayo Clinic team and the authors of this chapter. Other approaches may vary. Each patient is strongly encouraged to work closely with their medical team to develop the best approach for them

CT = computed tomography
PAVM = pulmonary arteriovenous malformation
PFTs = pulmonary function tests
ILD = interstitial lung disease

The sensitivity and specificity of each modality is variable and relative to the diagnostic value of the test. While PFTs are noninvasive and relatively accessible, they lack the sensitivity and specificity to identify early stages of ILD. A new ILD-Screen tool using age, height, total lung capacity, FEV1, diffusion capacity, and PFT indication demonstrated a sensitivity of 79% and specificity of 83% when validated with more

precise imaging [6]. Chest imaging is considered more sensitive but varies depending upon the technique used. While HRCTs have the best ability to identify early changes of ILD, they are associated with significant radiation exposure, as noted earlier; thus, questioning the long-term safety of repeat usage. They, however, do provide the best likelihood of identifying ILD with a sensitivity of 95% and specificity approaching 100% [7]. Chest x-rays on the other hand do not have the same sensitivity (80%) or specificity (82%) and diagnostic confidence is low (23%) [7].

Pulmonary function tests should include measures of FEV_1, FVC, FEV_1/ FVC ratios and DLCO estimates. We also obtain inspiratory and expiratory flow loops along with bronchodilator responsiveness, especially if the patient endorses hyperreactive airway disease like symptoms. Oximetry studies including the 6-minute walk test are carried out in patients with established ILD.

At Mayo Clinic, based on the age of the patient, smoking history (primary or secondary exposure), personal history of lung disease (e.g., asthma) and family history of pulmonary involvement, we consider performing a baseline HRCT with inspiratory and expiratory views. If there is no ILD, we follow patients clinically with PFTs and chest x-rays annually. We have a low threshold to obtain a HRCT in the event of decline in PFTs, cardiopulmonary symptoms or radiological abnormalities on the chest x-ray. In asymptomatic patients without exposure history or family history, a risk versus benefit discussion is warranted to evaluate patient's desire for more aggressive baseline assessment (HRCT) versus less sensitive or specific testing (PFTs and chest X-ray) (Figure 1).

If pulmonary findings are present, we refer our patients to an ILD Clinic to meet with Pulmonary experts and decide whether a bronchoscopy with transbronchial biopsies or open lung biopsy are needed for histopathological confirmation, or to rule out alternative etiologies. Occasionally, a bronchoscopy with bronchoalveolar lavage using an immunocompromised host protocol is needed to rule out atypical infections that could mimic ILD. Tissue biopsies are often not helpful if a strong diagnosis of a

telomere biology disorder can be made clinically or genetically (see also Chapter 3, Diagnosing Telomere Biology Disorders).

Individuals with TBDs can have pulmonary arteriovenous malformations (PAVMs) that can often mimic clinical features of ILD (see also Chapter 16, Vascular Complications). They may also be present in the absence of lung parenchymal involvement and should be suspected if there is a progressive decline in the DLCO without any change in the FEV$_1$ or FEV$_1$/FVC ratio [8]. Multiple diagnostic options exist though the most sensitive and specific is a transthoracic contrast echocardiogram (TTCE) or bubble echocardiography [9]. Injection of agitated saline while imaging the right and left ventricles allows visualization of microbubbles in the left ventricle that are not filtered out by the pulmonary vasculature suggest right-to-left shunting. An alternative and often less readily accessible evaluation may include a radioisotope (e.g. technetium-99m) ventilation/perfusion lung scan.

All individuals with TBDs should be informed of the pulmonary exposure risk with inhalants through recreational use and secondary exposure including nicotine (cigarettes, cigars, vaping) and inhaled marijuana. Counseling on cessation and referral to dependency centers for assistance with interventional and behavioral modifications is critical.

Cardiology

Little is definitively known on the association between TBDs and atherosclerotic cardiovascular disease (ASCVD) and associated coronary heart disease (CHD), myocardial infarction, angina pectoris, heart failure, stroke, transient ischemic attack, and peripheral artery disease. However, atherosclerosis has been demonstrated by senescence of vascular smooth muscle and inhibition of telomerase activity with marked telomere shortening, suggesting a potential for increased risk [10]. Additionally, observational studies of people without DC/TBDs suggest an association of short

telomeres with CVD and cardiovascular mortality including a 3-fold higher risk of MI and stroke [11].

Baseline assessment at diagnosis should include evaluation of blood pressure and blood cholesterol. Management of hypertension and hyperlipidemia should be in accordance with nationally recognized comprehensive care guidelines, including risk calculation and use of primary prevention where warranted [12, 13]. Androgen therapy, including danazol, increases the risk for hypertension, elevated LDL, and reduced HDL levels. A baseline lipid panel should be obtained prior to androgen treatment initiation and every 6 months while on therapy. Initiation of antihypertensives and lipid lowering therapy may be required though drug-drug interactions should be considered [14, 15].

Additionally, there is no clear data on the risk of congestive heart failure (CHF) in patients with TBDs. Data from individuals without TBDs suggest that cardiac myocyte telomere length is shortened in hypertrophic hearts, independent of age [16]. A baseline echocardiogram is not routinely recommended unless presenting symptoms warrant further investigation. Cardiopulmonary symptoms may manifest secondary to PAVMs as noted earlier and should be evaluated at baseline and throughout disease management when present.

Gastroenterology/Hepatology

Gastroenterology

Baseline evaluations should include physical assessment for the primary GI manifestations of TBDs, including esophageal stenosis, a celiac-like enteropathy, and B-cell immunodeficiency with enterocolitis (see also Chapter 17, Gastrointestinal Disease) [17]. Only if symptoms of esophageal stenosis are present (difficulty swallowing, intolerance of solids), should evaluation move forward with a video contrast swallow study (esophagram) or esophagogastroduodenoscopy (EGD). If stenosis is

present, intermittent balloon dilation and rarely stenting can alleviate symptoms. In the absence of symptoms, no routine evaluation is warranted.

Small intestine celiac-like enteropathy and immunodeficiency-mediated enterocolitis require exclusionary work up of other etiologies including infection or malignancies. Evaluation should include a celiac disease cascade; assessment of immunoglobulin levels; and quantification of T-cell, B-cell, and NK-cell subsets. Recommended diagnostic procedures include EGD and/or colonoscopy with biopsies [17].

Hepatology

The most common hepatic manifestations of TBDs include cryptogenic cirrhosis and nodular regenerative hyperplasia leading to non-cirrhotic portal hypertension (see also Chapter 18, Hepatic Complications) [18]. Baseline assessment of all patients include physical exam, family history, social history (alcohol and drug use), body mass index (BMI) and liver function test (Figure 2). In patients who are asymptomatic, lack family or social history concerns, have a normal BMI and liver function tests (LFTs), a Fibroscan (transient hepatic elastography) can be done at baseline and repeated every 2 years. If screening is positive, we prefer proceeding with magnetic resonance elastography (MRE) for evaluation of liver and spleen stiffness and anatomy. In patients with TBDs, the use of liver biopsy should be done judiciously, particularly if bone marrow failure and risk for infection and hemorrhagic complications are present.
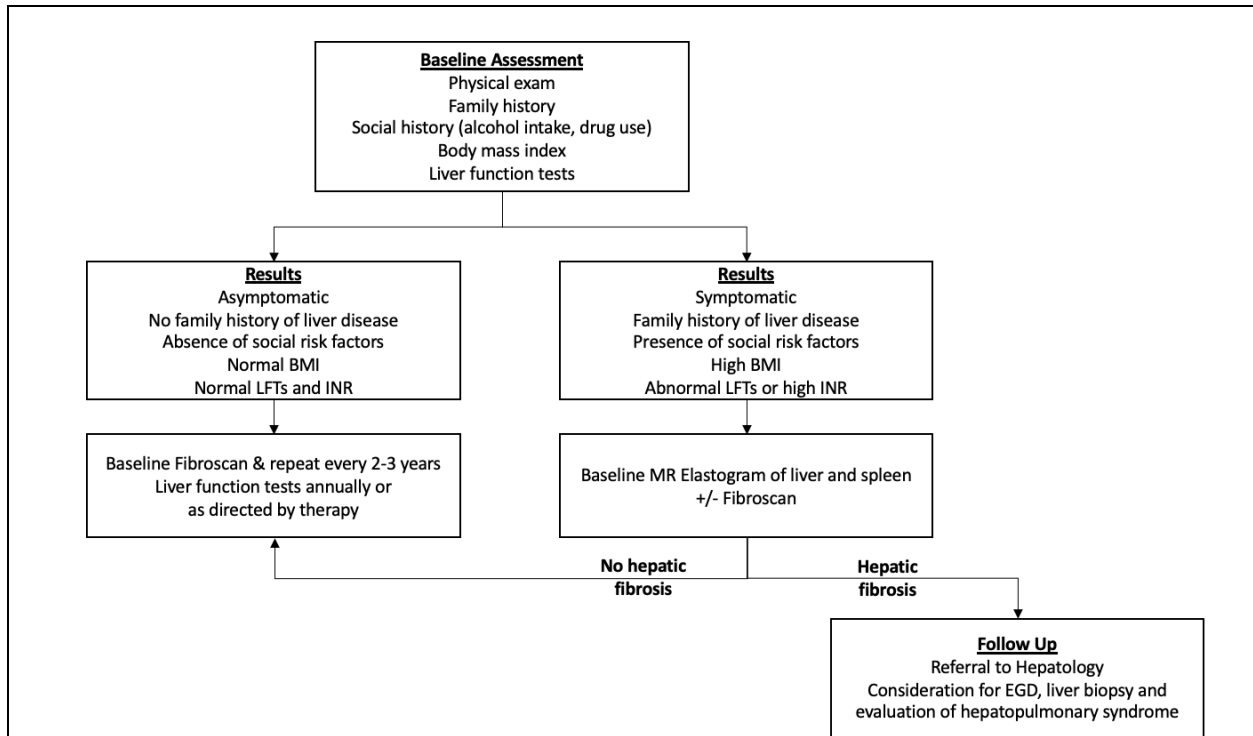
Figure 2: Hepatic function assessment in telomere biology disorders. These recommendations should be tailored for each specific patient in consultation with their medical team.

BMI = Body mass index
INR = International normalized ratio
PFTs = pulmonary function tests
EGD = esophagogastroduodenoscopy

If baseline assessments are negative, patients may revert to monitoring with Fibroscan every 2 years. If baseline assessment is concerning for fibrosis, we refer to Hepatology for consideration of EGD, evaluation and management of portal hypertension and assessments for hepatopulmonary syndrome. At times a liver biopsy may be necessary if there is clinical suspicion of portal hypertension with preserved synthetic liver function, absence of other causes for chronic liver disease and no risk factors for alcohol or non-alcoholic liver disease [18]. Meta-analysis of MRE as an imaging modality for liver fibrosis has demonstrated a sensitivity of 73% and a specificity 79% [19].

Patients on androgen therapy, such as danazol, should have their liver function tests evaluated pre-treatment and every 3-6 months while on therapy (or sooner if indicated).

Gynecologic Health

As described in detail in Chapter 20, Genitourinary Complications and Chapter 21, Gynecologic and Obstetric Considerations, females with TBDs may have specific gynecologic and genitourinary manifestations requiring close follow-up with a gynecologist. Annual routine gynecologic care is recommended, including cervical cancer screening with human papilloma viral (HPV) testing, beginning when they become sexually active or at age 21 years, whichever is first.

HPV vaccination is recommended for females <u>and</u> males between ages 9-27 years and is FDA approved up to age 45.

Endocrinology

There is no specific association with endocrinopathies and TBDs except for osteopenia and osteoporosis. We recommend comprehensive baseline screening of endocrinopathies including bone health with bone mineral density, 25-hydroxyvitamin-D total, thyroid hormone cascade, and diabetes mellitus assessment with fasting blood glucose and hemoglobin A1c if clinically appropriate. These tests are repeated annually or more frequently if clinically indicated.

In individuals of reproductive age, there is limited data on pregnancy outcomes. A recent cohort demonstrates an increased risk of progressive cytopenias, preterm and cesarean deliveries, and recurrent pregnancy loss in women with autosomal dominant inheritance [20]. Referral to a multidisciplinary center experienced in management of patients with hematologic conditions is recommended.

Orthopedics

Osteopenia and osteoporosis are not uncommon in individuals with TBDs (see also Chapter 22, Endocrine and Skeletal Disorders) [21]. Some data suggest that telomere shortening may induce osteoblast abnormalities associated with osteoporosis [22]. Thus, an adult individual with TBDs should have a bone density scan at baseline, and monitoring will depend on the findings. Vitamin D and calcium supplementation may be considered depending on findings.

Table 1. Screening recommendations for adult patients with Telomere Biology Disorders (TBD) by speciality.

Specialty	Evaluation	Recommendation
Cardiology	Hypertension	• Baseline blood pressure • Repeat vital signs with routine visits • Manage per nationally recognized guidelines *Patients on androgens (e.g.* danazol) *may warrant more frequent monitoring and treatment*
	Hyperlipidemia	• Baseline lipid panel • Manage per nationally recognized guidelines *Patients on androgens may warrant more frequent monitoring and treatment*
	Heart failure	• Address per symptoms

Pulmonology	Pulmonary fibrosis	• Assessment of baseline risk (see Figure 1)
		• Testing may include:
		  o Pulmonary function tests (PFTs)
		  o High-resolution chest CT with inspiratory & expiratory views
		  o Chest X-ray
		  o Early referral for shortness of breath or unexplained cough
		  o Nicotine dependency & secondary exposure counseling

	Pulmonary arteriovenous malformation	• Transthoracic echocardiogram with contrast (bubble echocardiography) • Nuclear medicine scan
Gastroenterology	Esophageal stenosis	• Esophagogram and/or upper endoscopy (esophagogastroduodenoscopy, EGD), if symptom • Medical management with balloon dilation and rarely stenting
	Enteropathy or Enterocolitis	• Imaging studies, EGD/colonoscopy with biopsies, and evaluation for infection and malignancy
Hepatology	Cirrhosis	• Assessment of baseline risk (see Figure 2) • Annual liver function tests including total/direct bilirubin, AST, ALT, alkaline phosphatase, albumin, PT, aPTT, and INR • Baseline transient elastography (Fibroscan ultrasound) • Magnetic resonance (MR) elastography if risk warrants • Referral to Hepatology for variceal assessment and liver biopsy as clinically indicated for concern of non-cirrhotic portal hypertension *Patients on androgens may warrant more frequent monitoring and treatment*
Hematology	Cytopenias; Aplastic anemia; Myeloid neoplasia	• Complete blood counts, reticulocyte counts and white blood cell differential at baseline • Bone marrow assessment at baseline: bone marrow aspiration and biopsy, flow cytometry, conventional and molecular cytogenetics, and myeloid panel by NGS for somatic variants
Immunology	Immunodeficiency	• Flow cytometry of peripheral blood lymphocyte subsets • Serum immunoglobulin levels (total and fractions)

Ophthalmology	Anterior segment	• Corneal assessment
	Posterior segment	• Retinal evaluation
	Adnexa	• Nasalacrimal duct, eyelid assessment
Orthopedics	Osteoporosis	• Bone density scan at baseline and then as needed

Table 2: Cancer Screening Considerations[‡]

Cancer Screening	American Cancer Society	United States Preventive Services Task Force	National Comprehensive Cancer Network
Breast Cancer	**Average** risk women screening mammogram: · 40 to 44 Y optional to start annual screening · 45 to 54 Y annual screening · 55 Y and older every other year OR yearly as long as health in good standing and live expectancy > 10 years Women should be familiar with how their breasts normally look and feel and should report any changes to a health care provider right away.	Biennial screening 50 to 74 Y The decision to initiate screening prior to 50 Y is individual.	**Average** risk women 40 Y and older, annual screening mammogram Risk assessment by 25 and counseling on benefits, risks, and limitations of screening. Clinical breast exams with provider visits. Individuals should be familiar with their breasts and promptly report changes to their health care provider.
Cervical Cancer	Initiate screening at 25 Y · HPV testing every 5 Y until 65 Y (preferred) · Cytology + HPV testing every 5 years · Cytology alone every 3 years	Initiate screening at 21 Y Cytology along 21 to 29 Y Age 30 to 65 Y: · HPV testing every 5 Y · Cytology + HPV testing every 5 years · Cytology alone every 3 years	None

Colon and Rectal Cancer	**Average** risk people start screening at 45 Y with a stool-based test or visual exam. Screening until 75 Y; The decision to screen between 76 and 85 Y should be individualized. Over 85 Y no screening required. Test examples: · Highly sensitive fecal immunochemical test every year · Highly sensitive guaiac-based fecal occult blood test every year · Multi-targeted stool DNA test every 3 years · Colonoscopy every 10 years · CT colonography every 5 years · Flexible sigmoidoscopy every 5 years	Recommend age 50 to 75 Y (Grade A); Recommend 45 to 49 Y (Grade B); Recommend 76 to 85 Y, based on patient's overall health, prior screening, and preference (Grade C) Test examples: · High-sensitivity guaiac fecal occult blood test or fecal immunochemical (FIT) test every year · Stool DNA-FIT every 1 to 3 years · Computed tomography colonography every 5 years · Flexible sigmoidoscopy every 5 years · Flexible sigmoidoscopy every 10 years + annual FIT · Colonoscopy screening every 10 years	**Average** risk people start screening at 45 Y with a stool-based test or visual exam. Screening until 75 Y; The decision to screen between 76 and 85 Y should be individualized. Test examples: · Highly sensitive fecal immunochemical test (FIT) every year · Highly sensitive guaiac-based fecal occult blood test every year · Multi-targeted stool DNA test every 3 years · Colonoscopy every 10 years · CT colonography every 5 years · Flexible sigmoidoscopy every 5-10 years
Lung Cancer	Currently undergoing revision; recommend USPSTF guidelines	Low dose CT screening in adults 50 Y to 80 Y with a 20 pack-year smoking history and *currently smoking* OR *have quit in the past 15 years*. Screening may be discontinued once a person has not smoked for 15 years, develops a life limiting illness, or is no longer willing to undergo lung surgery..	Low dose CT screening in adults [3] 50 Y with a 20 pack-year smoking history

Prostate Cancer	**Average risk, begin at 50 Y** if expected to live at least 10 more years. Screening modality: prostate specific antigen (PSA) blood test; digital rectal exam, optional (DRE). · If PSA < 2.5 ng/mL may only need to be retested every 2 years. · If PSA 2.5 ng/mL or higher, screen yearly.	Age 55 Y to 69 Y, individual decision in concert with provider. Screening modality: prostate specific antigen (PSA) blood test	**Average risk, begin at 45 Y** Screening modality: prostate specific antigen (PSA) blood test; digital rectal exam, optional (DRE). · If PSA < 1 ng/mL repeat testing at 2 to 4 year intervals. · If PSA 1-3 ng/mL, repeat testing at 1 to 2 year intervals.

‡: This table is reflective of select guidelines and statements from those guidelines have been selected to articulate standard situations. It is not meant to reflect the guidelines in their entirety and nor do they reflect all guideline options. Clinicians are encouraged to review these documents in their entirety when making clinical decisions for patients with TBDs/DC.

Table 3: Cardiovascular Disease Screening[†]

	United States Preventive Services Task Force
Hypertension	Begin screening at 18 Y with office blood pressure measurement. Confirm with home measurement before starting treatment. Screen every 3-5 years for adults 18-39 Y who are not at increased risk and have a documented normal blood pressure. Screen annually in adults > 40 Y and adults with increased risk.
Diabetes mellitus type 2	Offer screening to adults 35 Y to 70 Y who are overweight or obese
Hyperlipidemia	Screen initially at age 20 for familial disorders; Resume screening no later than 40 years of age. Screen approximately every 5 years or as clinically indicated

[†]: This table is reflective of United States Preventive Services Task Force recommendations. It is not meant to reflect the recommendations in their entirety and nor do they reflect all available consensus guidelines. Clinicians are encouraged to review any guideline in its entirety when making clinical decisions for patients with TBDs/DC.

Table 4: Vaccination[§]

	Ages 21 to 39 years	Ages 40 to 49 years	Ages 50 to 64 years	Age ³ 65 years
COVID-19	Recommended for everyone, follow schedule and doses per vaccine brand and current CDC guidance.			
Influenza	Recommended annually for everyone, inactivated or recombinant vaccine recommended for immunocompromised.			
Pneumococcus (PCV13 and PPSV23)	**Immunocompromised**: 1 dose PCV13 followed by 1 dose PPSV23 at least 8 weeks later, then another dose PPSV23 at least 5 years after previous PPSV23; at age 65 years or older, administer 1 dose PPSV23 at least 5 years after most recent PPSV23			**Immunocompetent**: PPSV23 1-time dose. May consider PCV13 in shared decision making effort.
Tetanus diphtheria acellular pertussis (Tdap and Td)	1-time dose of Tdap, then boost with Tdap or Td every 10 years			
Human papilloma virus (HPV)	**Immunocompetent**: Initiate series for adults up through age 26 years, consider series initiation for ages 27 through 45 based on shared decision making **Immunocompromised**: Initiate series for adults up to age 45 years.			
Herpes zoster or shingles (HZV)			2 doses of Shingrix (regardless of previous Zostavax) at age ³ 50 years unless contraindicated	
Meningococcal Serogroup B Meningococcal conjugate	**Immunocompetent:** Meningococcal conjugate vaccine (MenACWY) single dose + Meningococcal vaccine serogroup B (MenB) 2 or 3-dose series based on shared decision making. **Immunocompromised:** Meningococcal conjugate vaccine (MenACWY) 2-dose series, revaccinate every 5 years + Meningococcal vaccine serogroup B (MenB) 2 or 3-dose series every 2 to 3 year, based on risk re-evaluation.			

§: This table is an abbreviation of the CDC recommendations for vaccinations. Guidelines are subject to change and update. Please refer to the full guidelines noted below for details and the most up to date guidance on vaccinations.

Guidelines

United States Preventive Services Task Force Recommendations:
https://www.uspreventiveservicestaskforce.org/uspstf/recommendation-topics

American Cancer Society Guidelines:
https://www.cancer.org/healthy/find-cancer-early/american-cancer-society-guidelines-for-the-early-detection-of-cancer.html

National Comprehensive Cancer Network Guidelines:
https://www.nccn.org/guidelines/category_2

Center for Disease Control and Prevention Vaccinations Schedules:
https://www.cdc.gov/vaccines/schedules/index.html

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Chapter 28

Diet, Wellness, and Prevention

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Introduction

Although telomere biology disorders (TBDs) such as Dyskeratosis Congenita (DC) are clearly inherited, it is natural for patients and caregivers to inquire about whether lifestyle factors such as diet can influence the progression or expression of telomere disorders. When any person is confronted with a diagnosis over which they have very little control, it is an expected and natural response to want to take as much control over the situation as possible. Because lifestyle is a potentially modifiable factor in many diseases, it is common for individuals to inquire about how integrative practices such as diet, supplements, or physical activity might impact their disease. Unfortunately, predatory marketers (such as "wellness" and dietary supplement companies) can use this time of vulnerability to prey on the fears and hopes of patients, oftentimes promoting un-proven and expensive telomere lengthening supplements or diets. Conversely, if a patient is motivated by their diagnosis to make positive lifestyle changes that may impact their overall health in a positive way, we want to encourage and support them using the best available evidence.

Telomeres and Lifestyle

To date, there are a small but growing number of studies examining the role of diet and physical activity on telomeres. Because telomere shortening has been associated with inflammation and oxidative stress, lifestyle factors known to mediate these factors have been the primary focus of study so far [1-4]. Results have been mixed, with some studies showing no benefit and others showing modest benefit on either telomere length or telomerase activity. Several recent meta-analyses have been helpful in summarizing these studies of diet and/or physical activity on telomeres [1-3]. Most studies are of very small sample sizes of healthy subjects (without known TBDs), they are primarily cross-sectional or cohort studies, and comparatively few are randomized controlled trials [1-3]. It is important to put the subject type, study size and quality of these studies in context when speaking with patients and caregivers about the impact of lifestyle directly on telomere length or activity.

There is a much larger body of evidence that relates to modifiable lifestyle factors and prevention of cancer. Because individuals with TBDs have a higher risk of developing cancers, it is advisable to review the known links between dietary and physical activity patterns and cancer prevention. Many of the known, well-studied lifestyle changes to promote cancer prevention overlap significantly with the emerging telomere diet research [5, 6]. For these reasons, helping patients to adopt lifestyles that promote cancer prevention may have the most impact in terms of modifiable disease factors and overall health.

Telomere-Specific Diet and Physical Activity Research

In recent years there have been several published reviews or meta-analyses of the literature related to diet and/or physical activity and telomere length or telomerase activity. The range of studies included in these reviews include dietary patterns known to be anti- or pro-inflammatory as well as specific nutrients such as vitamin D or fish oil. Please refer to the individual reviews for a summary of their study inclusion criteria and

tables of summary findings [1-4]. Again, it is important to remember that the overall size and type of studies can only suggest risk or protective associations and not causality of telomere changes.

The most recent review from Navarro-Ibarra and colleagues published in 2020 includes fifty studies published through 2018 [1]. Contrary to a previously published review by Perez and colleagues in 2017 which showed no overall effect of diet on telomere length, the updated Navarro-Ibarra review showed some correlations with diet, activity and telomere length or telomerase activity. The mechanisms of such an effect are not fully understood in the context of telomeres, but the assumption is that pro-inflammatory diet components are associated with telomere shortening whereas anti-inflammatory diet components are associated with maintaining telomere length and telomerase activity. Of note, some studies showed that increased serum concentrations of vitamin D or antioxidants had positive correlations with telomere length, but the impact of increased consumption of these nutrients through food or supplements was not independently evaluated.

In general, dietary patterns that included a higher consumption of plant foods were associated with an increase in telomere length. Most studies showed that dietary patterns such as the Mediterranean Diet and those that include more antioxidant components such as those found in vegetables and fruits, whole grains, seeds, and walnuts were associated with longer telomeres. Conversely, diets that included more pro-inflammatory components such as sugary beverages and processed meats were associated with telomere shortening.

Of the twenty studies in the Navarro-Ibarra review that evaluated some aspect of physical activity and telomere length, all studies showed a positive association between moderate physical activity and telomere length.

Cancer Prevention Diet and Physical Activity Research

Individuals with TBDs have higher frequencies and develop cancers at younger ages than would be expected and are considered "cancer-prone" (see Chapter 9, Solid Tumors). Therefore, encouraging modifiable lifestyle factors that are known to be protective against cancer development and recurrence may be even more important for those with TBDs than for the general population. Not surprisingly, emerging findings from the small amount of published lifestyle and telomere research also aligns closely with the much larger body of evidence related to lifestyle factors and cancer prevention. For this reason, it may be most practical for providers and patients to look to the large amount of resources developed for promoting cancer prevention through modifiable lifestyle changes.

The most recent Guidelines for Diet and Physical Activity for Cancer Prevention were published by the American Cancer Society (ACS) in 2020 and include a variety of specific recommendations for the prevention of cancer [5]. While the published guidelines are written for the physician and public health audience, the information contained in the guidelines becomes the scaffolding on which most evidence-based guidelines and patient resources are built.

The American Institute for Cancer Research (aicr.org) also integrates cancer prevention research globally into guidelines, and in 2018 published their most recent *Third Expert Report* [6] and includes similar and expanded information as compared to the ACS. The mission of the AICR is to "…*champion the latest and most authoritative scientific research from around the world on cancer prevention and survival through diet, weight, and physical activity, so that we can help people make informed lifestyle choices to reduce their cancer risk.*" A result of this mission includes a breadth of educational materials online to help individuals put these research derived evidence-based guidelines into practice every day.

American Institute for Cancer Research (AICR)

How to Prevent Cancer: 10 Recommendations

1. Be a healthy weight
2. Be physically active
3. Eat a diet rich in whole grains, fruits, vegetables and beans
4. Limit consumption of fast foods and other processed foods that are high in fat, starches, or sugars
5. Limit consumption of red and processed meat
6. Limit consumption of sugar-sweetened drinks
7. Limit alcohol consumption
8. Do not use supplements for cancer prevention
9. For mothers: breastfeed your baby if you can
10. After a cancer diagnosis, follow these recommendations if you can

Messages and Resources for Patients

A significant diagnosis such as a telomere biology disorder can be an overwhelming time for patients and their loved ones. It is expected that patients will have questions about their role in the development, progression, or expression of their disease. These questions may very likely include questions about diet and lifestyle. Although it is sometimes uncomfortable for medical professionals to discuss topics that do not have clear evidence, it is important that we provide patients with *what we do know* – otherwise bad actors with less good intentions may attempt to fill that information void - and not always in the patient's best interest.

Some talking points to consider:

- **Validate the importance of patient locus of control concerns:** *"This is big and unexpected news, and it's natural for you to wonder how your actions might have*

contributed to this diagnosis. It's important to know that developing a TBD is related to your genetics, and we don't have any evidence that how you have lived your life contributed to this diagnosis."

- **Put the number and quality of research studies related to telomeres and lifestyle into context:** *"There are more studies about the role of nutrition and lifestyle being published every year. In fact, you may read about single studies in the popular news media because telomeres are a hot topic in science and medicine. These studies are done in people who have normally-aging telomeres, and not TBDs. It's important to understand that compared to what we know about the biology of telomeres, the amount of information that we know about lifestyle and telomeres is much smaller and less clear. There are some small studies done on healthy subjects (persons without TBDs) that may start to give us some insight into whether or not lifestyle modifications can alter telomeres in patients like yourself – but so far there is no evidence that diet, supplements or lifestyle will definitively change the length of your telomeres. Our group is interested in every way that we can help you and we will continually be re-evaluating the research as it is studied and published."*

- **Give the patient broader context for adopting healthy lifestyle changes:** *"One of the things we know about patients with TBDs is that you have a higher likelihood of developing cancer. There are several lifestyle changes that all of us can make to help prevent cancer. The good news is that these are things that can benefit you and everyone around you – whether or not they have a TBD. Also, the same recommendations that we know can help prevent cancer are many of the same things that some of those smaller studies about telomeres and lifestyle have found – so it's possible it may be even more beneficial for you, and there are no health risks to following a cancer preventative diet."*

Online Resources

Online evidence-based resources related to diet, lifestyle, and cancer prevention:

- American Institute for Cancer Research (AICR): https://www.aicr.org/cancer-prevention Provides information for providers and consumers, including detailed information about diet, foods that fight cancer, food facts, recipes, and information on supplements.
- Consumer-facing summary of ACS 2020 Guidelines on Diet and Physical Activity for Cancer Prevention: https://www.cancer.org/healthy/eat-healthy-get-active/acs-guidelines-nutrition-physical-activity-cancer-prevention/guidelines.html
- *Eat Right to Fight Cancer* information from the Academy of Nutrition & Dietetics Oncology Nutrition Dietetic Practice Group includes, FAQs, recipes, menus and links to more resources: https://www.oncologynutrition.org/erfc
- Food and Cancer Risk information from the American Society of Clinical Oncology (ASCO): https://www.cancer.net/navigating-cancer-care/prevention-and-healthy-living/food-and-cancer-risk
- National Center for Complimentary and Integrative Health (NIH) provides great information on evaluating evidence of complimentary and integrative health to become a better informed consumer: https://www.nccih.nih.gov
- Mediterranean diet ideas and recipes from Mayo Clinic:
  - https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/mediterranean-diet-recipes/art-20046682
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Chapter 29

Navigating Telomere Biology Disorders

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Introduction

When faced with the diagnosis of a rare illness, one of the greatest challenges is understanding what you can and cannot control. Not only do you not know what you are dealing with, but more often than not the medical care providers are also at a loss. Everyone responds to this situation in different ways. This chapter is designed to help you navigate this complicated disease, advocate for yourself or your loved one, and care for yourself and other members of your family to sustain yourself for the journey ahead.

> *DC is part of my life, but DC does not define me.*
>
> —Young adult with DC

A diagnosis of Dyskeratosis Congenita (DC) or a Telomere Biology Disorder (TBD) comes with a complexity of concerns. Living with a rare diagnosis, with an illness course that is not fully understood and can vary greatly from person to person, is filled with layers of uncertainty. A challenge for many people newly diagnosed with DC/TBD is how to embark on a journey with an uncharted path and few fellow travelers. The ever-evolving scientific landscape and a committed cohort of scientists and clinicians afford hope and great promise for the treatment and management of DC/TBD.

Whether it is regarding the "triad" of DC (i.e., abnormal nails, skin pigmentation, and oral leukoplakia), short stature, bone marrow failure, lung disease, cancer, etc., the clinical characteristics of DC can cause considerable discomfort, require regular attention, and impact one's well-being, self-esteem, and quality of life. Those things that cannot be seen (e.g., low blood counts, fertility issues, liver disease, etc.) may dictate many aspects of the life of a person with DC.

Depending on one's coping style, adapting to an illness with multiple, varied manifestations can create unprecedented anxiety. Not knowing what the road ahead holds can be immobilizing and can create self-imposed limitations. There is the possibility of pulmonary fibrosis, liver disease, cancer, and other life-limiting complications. In addition, the need for bone marrow or solid organ transplantation, or other serious intervention, may be looming. Coping with DC involves learning to adapt to those future possibilities, but not becoming overwhelmed by them. Part of coming to terms with DC/TBD is about acquiring the knowledge to manage the illness, and not having the illness manage you.

When families facing DC/TBD come together, a subtext is revealed: regardless of what is shared in common, there are also many differences. Each person's story is their own,

adding to the emotional complexity of DC/TBD. There can be comfort in the differences as well. Uncertainty is cumbersome emotionally but also holds out hope as one of its components.

The Emotional Journey

> *When I was diagnosed, I wished it was breast cancer, because it would be something people knew, understood, and for which there were specific treatments.*
>
> — Adult with DC/TBD

Although all life-threatening illnesses can cause an individual or family to feel isolated, isolation is more "prevalent" with less "prevalent" diagnoses. The availability of illness-specific organizations can dramatically change the experience of the illness. Team Telomere is that organization for families affected by DC/TBD.

Team Telomere, formally Dyskeratosis Congenita Outreach, was started in 2008 when researchers from the National Institute of Health, including Dr. Blanche Alter and Dr. Sharon Savage, brought together patient families for support and to help research move forward. The premise, set by our founder Nancy Cornelius, was that no one is ever alone. In the years since the forging of the organization, the mission has always remained consistent.

Individuals living with chronic, life-limiting illness develop different coping styles. One can be proactive, coping assertively by choice. For example, one can choose to compensate for limited knowledge about DC/TBD by seeking out all possible relevant medical information. This style may not work for everyone. Some people may choose to know less, managing things as they happen; others may try to ignore the illness altogether. Some will choose to identify someone in their life to become the "keeper" of the most up-to-date clinical information.

The type of "information seeker" you are will influence how you proceed to gain knowledge about DC/TBD and what you do with that knowledge, whether you are an individual with DC/TBD or a family member. In more common illnesses, even a passive person can be inundated with information from media, colleagues, friends, and family. With information not overly abundant regarding DC/TBD, you have to be willing to seek out knowledge.

> *Part of the barrier of a rare disease is that throughout the journey you always feel alone.*
>
> — Parent of a child with DC

The rarity of the illness and the wide range of symptoms do not make DC/TBD easy to diagnose. In many cases, symptoms precede the diagnosis for an extended period of time. Knowing that something is wrong but being unable to quantify it can create uneasiness, loneliness, an ongoing sense of anxiety, and questioning oneself. Even after the diagnosis is established, that period of uncertainty may have established a pattern of coping that persists.

Another common challenge of DC/TBD is having an illness that is difficult to explain to others. Until recently, most people had not heard of a telomere. Telling someone that you have short telomeres does not automatically engender resounding support or compassion.

> *Because it was unknown, people did not know how devastating, even fatal it was.*
>
> — Adult with DC

The Role of the Advocate

To be an advocate for somebody who cannot advocate for themselves, or a patient who is fully occupied with the challenges of their disease and treatments, can be difficult in many ways. This is often the case with DC/TBD; a very few truly understand the disease and what it means to each individual can vary drastically. Engaging with resources upon diagnosis is critical; these can come in many forms. Teaming with medical providers and becoming a partner in care is vital. Some things to consider when advocating for your loved one include:

- **Patient advocacy organizations** such as Team Telomere offer support in the forms of regional access, financial assistance, connection to specialists familiar with the illness, and community connections.
- **Social workers** offer support and mediation between medical/mental health teams, insurance, government agencies resources and family members. Ask the center where your DC/TBD patient is being treated for a referral to a social worker.
- **Palliative care**, often misunderstood, offers support and comfort to the entire family when a loved one is living with a serious illness. It's never too early to start learning about the services and additional support palliative care can provide, as current treatments and plans for care continue. Ask the center where your DC/TBD patient is being treated for a referral to palliative care.
- **Education advocates** have knowledge of your region's educational laws, e.g., 504 plans and individual education programs (IEPs). Note that these can extend through the college years. Ask your child's school for a referral to an education advocate.

Identifying and caring for these relationships is the responsibility of all parties involved. Coming from a place of partnership is key, with collaboration as the focus for the best interest of the individual with the disease.

Staying organized throughout your loved one's medical journey is critical. You will likely be inundated with information from numerous sources, and staying organized can provide a sense of control over the situation. Create an organizational system that works for you to keep track of conversations, medical records, contacts at various centers, next steps, etc. This could be in a binder or notebook, or it could be an electronic record (e.g., Google Docs).

Nobody knows you or your loved one better than you. Never be afraid to ask questions, send inquiries, or be made to feel that you've done something wrong. DC/TBD research and discovery are currently progressing very quickly, and what we knew three years ago seems light years behind us at times. Treatments and research move forward because of those that work for the betterment of individuals with DC/TBD.

Caring for the Caregiver

Being a caregiver creates tremendous demands on the mind, body, and spirit. In line with Team Telomere's founding belief that no one is ever alone, caregivers are encouraged to do whatever they can to care for themselves during what may be a protracted medical journey that is more of a marathon than a sprint.

- **Take time for self-care.** Caregiver burnout leads to patient suffering. Remember the mantra shared by one nurse caring for a DC/TBD patient, "You cannot serve from an empty vessel." Another expression that especially resonated with a parent of a DC/TBD patient is, "Put on your own life jacket first."
- **Stay connected to friends and family.** Do not be afraid to ask for help. One way to visualize a support network is to imagine concentric circles with the patient at the center, the primary caregiver in the next outward circle, supporters of the caregiver in the next outward circle, and so on. Anyone in these circles can lean outward for support. Sometimes well-intentioned supporters make generic offers like, "Let me know what I can do to help." In response to this, many patients and caregivers have reported that it is most effective to request help with very

specific and defined activities, such as providing meals or child care on a certain day of the week or driving the patient to and from scheduled medical appointments.

- **Care for your body in ways that nourish you.** Walking outdoors, exercise, yoga, meditation, warm baths, aromatherapy, reading a favorite book, sleep, hobbies, and taking time to prepare and eat a healthy meal are all examples of self-care.

- **Connect to others sharing the same or similar experiences.** Look to support groups at the center where the DC/TBD patient is being treated and/or through Team Telomere.

- **Mental health specialists** with expertise in illness and grief and/or supporting caregivers can be tremendously supportive. Ask the center where the patient is being treated for referral to a mental health specialist.

- **Gather daily inspiration.** As you go through a long medical journey, along the way you will likely run across quotes, images, text passages, and comments from people you interact with that are especially meaningful and impactful. Collect these in a notebook, Google Doc, or whatever storage format works for you, and continue to refer to these to remind yourself of your goals, vision for success, and the support network around you.

Dyskeratosis Congenita in Families

Attending the DC family meeting allowed us to reconnect as a family.

— Parent of a child with DC

DC/TBD impacts the whole family. The number of affected family members and their ages will influence the emotional profile and needs of a family. The inheritance pattern of DC/TBD presents an emotionally complex story, with the illness affecting multiple generations in some families. The fact that a grandparent, parent, and child can be

dealing with DC/TBD at the same time, or that some can be lifelong asymptomatic carriers, adds to the multiple layers of complexity.

> *I knew I had what my father had.*
>
> — Adult patient with DC

Prior to diagnosis, living without knowing what is wrong, but in some cases seeing that you and other family members share similar symptoms, can be unnerving. Alternatively, it can cause family members to believe that those symptoms carry less significance, because they seem more commonplace when they present in more than one family member. The diagnosis is complicated, both for those in the family who are affected and for those who are not.

> *I have learned that the 'non-affected' family members are impacted as much by my diagnosis as I am.*
>
> — 33-year-old adult with DC

Parents' Journeys

When a child is diagnosed with DC/TBD, orchestrating their medical care, sustaining family life, managing responsibilities and family finances, all while maintaining hope, falls to the child's parents. They must explore and assimilate tremendous amounts of information to stay the course.

The diagnosis itself presents an emotional crisis. It may take time before parents can move from shock and disbelief to a more proactive mode of coping. Many parents feel anxious or depressed upon learning the diagnosis, unsure of what to expect. However, finally having a diagnosis may alleviate some of the existential anxiety which develops when you know something is wrong, but you do not have an answer. The ability to

contain the anxiety, manage the emotions, make decisions, enjoy life, and continue to function, are all skills to be mastered.

If a marriage was previously stressed, difficulties in the relationship may be further exacerbated by the illness. However, in some situations, couples may feel that the strain and the magnitude of the issues they face enable them to become stronger together. Individual parents may cope differently. One parent may need to learn everything there is to learn to plan strategically for the future, whereas the other may choose to stay focused on the moment at hand. One parent may need to talk and to cry, while the other may appreciate silence. Differences in coping styles may relate to gender, culture, age, and personality, and should be acknowledged so that each parent can be supported for their strengths, insight, and ability to adapt during the course of the illness.

Depending on parents' ages at the time of the child's diagnosis, or that of an adult with DC/TBD, the implications for the family are great with regard to having more children. The increased success and refinement of preimplantation genetic diagnosis (PGD) and surrogacy options present methods that can be utilized to conceive a child to be a matched donor for stem cell transplantation for the child with DC/TBD, or for someone with DC/TBD in choosing to have an unaffected child.

Assisted reproduction can be physically, emotionally, and financially draining. Unsuccessful PGD attempts may serve to delay having more children and can create other conflicts. This phase can be an emotional one in the life of a DC/TBD family, as treatment options, as well as additional children, stand in the balance. Successful PGD attempts can set the course of a family towards having a baby and planning a stem cell transplant, creating an unusual dichotomy: anticipating the birth of one child and the transplant of another. Whether you have DC/TBD yourself or have a child with DC/TBD, actively choosing to have a child without DC/TBD can present an existential crisis within the family.

The Journey for Children with DC/TBD

Even though we all have DC children, they are all different.

— Parent of a child with DC

Physical and other differences may set children with DC/TBD apart from their peers and can be factors that cause children to feel isolated, lonely, or depressed, affecting their self-esteem and ability to focus on age-appropriate achievements. Counseling and meeting others in similar situations can be a great benefit. Children also need to feel that they can confide in their parents, their medical team, and important people in their lives when they feel limited physically or emotionally by DC/TBD.

How parents accept and face the illness will influence how children with DC/TBD develop and adapt to it. If parents can create an environment that allows for dialogue, children will find it easier to ask them questions about their illness and treatment and become more active participants in discussions about DC/TBD and its management. This is true whether it is the adult or the child who has DC/TBD (or both).

Children often know much more about DC/TBD than adults might believe. In addition to what they have been told, they have independent interactions with professionals and other children while in the hospital and may also overhear information from ambient conversation. Children tend to be good regulators of their own knowledge base, providing insights about what they know and what they want to know.

Children of all ages and all stages of illness need to be allowed to continue to grow, regardless of the status of their medical condition. Maximizing their capacity and recognizing achievements of all magnitudes will enable the development of emotional strength and support continued growth.

At any age, the educational environment may present unique issues for children with DC/TBD. Upon entering school, children begin to see themselves in comparison to other children. School may be where they learn that everyone does not have DC/TBD or a family member with DC/TBD, not everyone has so many doctor visits, needs blood drawn frequently, or takes medication. This age becomes a time of further inquiry and therefore presents an opportunity for greater understanding and growth. If a child is sick and unable to attend school, if they are unable to participate in activities because of their physical ability or limited stamina, or if they are perceived as different from their peers, they may begin to feel depressed or emotionally uncomfortable in a manner that they may not have experienced previously.

Children need support learning how to adapt, respond, and connect to their peers around matters related to DC/TBD. As school-age children grow, they begin to differentiate themselves from their families and develop increasingly strong relationships with their peers. Physical limitations, or particular treatments (e.g., bone marrow transplantation) may influence a child's social activities and relationships. Each child will need help finding a balance between social and family relationships in the context of living with DC/TBD, allowing the child to feel nurtured while gaining a sense of independence.

Development of a child's sense of self and how that relates to their illness will be influenced by the age and developmental stage at which they learn about their diagnosis. A frequent concern for parents is what and when to tell children about DC/TBD. At each stage of development, children need age-appropriate explanations of the condition and its required treatment. Such information should grow in sophistication as the child grows. Developmentally appropriate explanations and access to information throughout the illness experiences enhance the child's ability to understand and deal with DC/TBD.

As children get older, they begin to assent, consent, and participate in decision-making about their own care. As their children become more active decision makers, parents

may feel some relief that they are now making decisions with, rather than for, their children. At the same time, parents may feel uncertain about their child's decision-making skills compared to their own. Some parents have expressed anxiety about how their children will learn to make sophisticated, well thought out, difficult decisions for themselves. Of equal concern for parents is whether their child will continue to include them as an integral component of their medical care. Taking care of a child's illness is a job that no parent ever wants in the first place, but alternatively, once proficient at it, it is not a job that many parents want to give up.

Additional guidelines for children can be found in Chapter 25, Routine Healthcare for Children with Telomere Biology Disorders.

Adolescents

As children with DC/TBD mature towards adulthood and begin to take responsibility for their actions, the concomitant challenges of age-appropriate development (complete with inappropriate choices) do not evade them. Adolescents have the capacity to understand DC/TBD in greater depth and may need assistance as they work to integrate this knowledge into their daily lives. For adolescents, challenging "the system" is age-appropriate and functional at times, facilitating emotional growth and allowing them to assert themselves as individuals. It can be expected that even those with the mildest of temperaments may rebel against the "rules" of DC/TBD. Adherence to medical regimens may be incomplete and should be given particular attention at this age. Risk-taking behaviors that relate to peer pressure, including illicit drugs, alcohol use, and sexual activity are all components of the adolescent's developmental landscape.

Additional guidelines for adolescents can be found in Chapter 26, Transitioning from Pediatric to Adult Medical Care.

Growing to and Through Adulthood

Many individuals with DC/TBD, who have lived with the knowledge of their illness since childhood, will continue to make decisions as young adults in collaboration with their parents. Having grown up in a "medical partnership" with their parents, they have grown accustomed to having them involved in their medical care. Growth for the individual with DC/TBD can also become a time of growth for other family members. Parents will sometimes need assistance in strengthening their skills at enabling their "aging" children to become responsible for their own care. In turn, parents are crucial in educating and empowering their children, while learning to trust them and their judgment. As with growth in all facets of life, there can be occasional dissonance between parents and children living with DC/TBD. Ultimately, parents will need to learn to support and appreciate their grown children's choices. Newly emerging adults with DC/TBD will need to learn to trust and engage their families at times of crisis and when they need assistance.

Young adults may find themselves torn between the desire to be proactive about their health and their desire to fit in socially. This struggle can be exacerbated by the complexities of their emotional journey with DC/TBD, combined with a sense that life may be on an accelerated path. As those with DC/TBD age and medical problems emerge, groundwork set in earlier years will encourage them to rely on health care providers for treatment and support.

Finding their own voices, taking responsibility for managing their own illness, becoming primary decision makers while using their parents as partners or consultants, and truly becoming independent, are appropriate and very significant steps for young adults. It is important to help individuals with DC/TBD gain their independence, while helping them understand that they can still rely on their families for information, support, assistance, and guidance. Medical partnerships with parents should be well established before children age out of pediatric care.

Becoming a young adult leads to a more comprehensive understanding of DC/TBD and new intellectual and emotional realizations. Issues that may have otherwise been dormant at other developmental stages will need to be addressed. Young adults who face the most severe manifestations of DC/TBD may, of necessity, remain more physically and emotionally dependent on family members. At each stage, issues of dependence and independence may need to be negotiated.

Becoming a young adult carries with it certain responsibilities for all individuals, even more so a person with DC/TBD. Becoming responsible for one's own medical care begins in earlier stages of development as an individual learns about DC/TBD. Being in charge of one's medical care is best seen as a partnership between the person living with the disease, members of that individual's support network, and the medical team. Taking care of oneself does not mean having to deal with DC/TBD alone.

Growing up with DC/TBD, establishing and mastering life goals, forming relationships and dealing with issues of partnership, sexuality, marriage, children, financial and insurance concerns, while managing a complex illness, organizing a variety of medical specialists in the interest of your care, and dealing with potential medical risks, present unique challenges for adults living with DC and their family members. Adults who have DC/TBD and are the parents of a child with DC/TBD have to negotiate their own medical issues and concerns as they anticipate and take care of their child's needs. In such situations, as children with DC/TBD mature, they will be exposed to the medical trajectory of their parents or even grandparents, leaving them wondering if they will experience a parallel illness course.

Who you are in the world is often amplified by the friendships you create. Whom do you tell that you have DC/TBD, and what and when do you tell them? These are complex issues, as they seem to be inherently related to those you trust, combined with an ongoing evaluation of the relevance of who needs to know, and your sense of what they will do with the information. Each individual must decide how they will incorporate DC/TBD into the structure of their lives. This issue can frame early stages of

relationships with friends, roommates, and romantic partners. When someone who has DC/TBD embarks on a relationship, questions about the nature of the illness, as well as the personal implications for the person with DC/TBD and for the partner, emerge. The revealing of DC/TBD, the short version, and then DC/TBD, the long version, becomes a component of the "dating" process.

Once in a relationship, partners of individuals with DC/TBD may need an outlet for information, expression, and assistance. Aspects of DC/TBD may be understood intellectually, but it is only when a partner's condition worsens that some of the partner's own concerns may emerge. Negotiating the caregiver roles of partners and parents for the person with DC/TBD presents an additional developmental and emotional challenge.

Avenues for Support

> *We had never met anyone with Dyskeratosis Congenita before. We both arrived scared and nervous and are leaving excited and hopeful.*
>
> — Parents after the experience of a DC family meeting

Team Telomere has compiled a vast array of resources to help individuals with DC/TBD and their families. The website, newsletter, monthly calendar, chats, educational meetings, fundraising activities, and other events that bring people in the community together are invaluable in sustaining and inspiring hope, joy, and a sense of being connected. They clearly enhance the lives and access to medical care for persons with DC/TBD.

Team Telomere, in collaboration with Camp Sunshine, has hosted biennial sessions for families of children with DC/TBD since 2010. Additionally, there are other opportunities created by Team Telomere to bring families together. All such programs serve as vehicles to help educate and support those with DC/TBD and their families. Retreat-style, "campferences" or one-day family events blend educational sessions

presented by clinicians and researchers with psychosocial support and recreational activities have proven to be invaluable in empowering and inspiring hope in the lives of individuals with DC/TBD and their family members. This combination of education and the joy of community-based activities provides a successful formula to help meet the needs of families dealing with life-long illness.

Family Comments on Team Telomere Events

- *Camp Sunshine has given us hope for the first time in seven years.*
- *We are not alone.*
- *My son was transformed from being 'the boy with weird skin and nails' to being accepted and meeting two other kids his age.*
- *It gave us a directed path of information and help.*
- *We laughed for the first time in years, truly laughed.*

Siblings

> *Why did he inherit the DC? It could have as likely been me; the odds were the same.*
>
> —Sibling of a teen with DC

Siblings and sibling relationships are exceptionally significant, but may not always be the first priority in a family when a diagnosis of DC/TBD occurs, given the complex nature and the demands imposed by the illness and its treatment. Siblings of children with DC/TBD present their own unique concerns, some more and some less apparent. Siblings may feel guilty that their sibling was diagnosed and that they are healthy. In the case of genetic disorders like DC/TBD, these feelings are often further exaggerated. Non-affected siblings can experience complicated emotions at not having DC/TBD, fear and worry for their siblings, as well as normal sibling rivalry and then remorse at feeling jealous.

Siblings often use each other as reference points in life, defining themselves in relationship to their siblings. They see themselves in a comparative context: "I am one of three; I am the oldest; I have no brothers or sisters." DC/TBD becomes another defining parameter in the relationship and is potentially even further complicated if there is more than one child in the family with DC/TBD. Sibling relationships can be among the strongest bonds in life, needing to be maintained and nurtured. It is important that affected and non-affected siblings have the opportunity to talk with their parents and with each other.

Specific Issues for Siblings, from a Team Telomere Family Meeting

- Concern that all attention goes to the child with DC/TBD
- Feelings of neglect or isolation, less loved
- Never meeting or seeing others to relate to in day-to-day life (prior to regional or national gatherings)
- Guilt over not having DC/TBD
- Powerlessness, when a clear avenue of how to help is not apparent
- Wishing they could help more
- Needing to understand DC/TBD more
- Needing help in how to explain DC/TBD to their friends or peers
- Worry for the sibling
- Worry for the impact of DC/TBD on their own future family
- Not wanting to burden parents with their needs
- Wanting to be "good" so as not to create further issues in the family
- Feeling responsible for sibling and parent's well-being

> *I was diagnosed after my brother's autopsy results came back.*
>
> — DC patient and sibling

Siblings, whether affected or unaffected, worry about each other and themselves. Depending on the situation, siblings can exhibit emotional responses to the illness equal to or stronger than those who are affected. Anxiety is a dominant emotion experienced by children with DC/TBD and their siblings. Siblings, even understanding the magnitude of their sibling's illness, can still feel left out. Some may feel that they are less important to their parents because they do not have DC/TBD, or because they are not getting as much attention. Having such feelings, even if they are not verbalized, can cause a brother or sister to experience distress, so sometimes all of these emotions remain unspoken.

Open communication, education, and the opportunity to express and process experiences will enable siblings to find solace on the DC/TBD family journey. It is important to address unaffected children's feelings and questions, while including them in illness-related activities whenever possible. Siblings need their own time with parents, to have age-appropriate explanations of DC/TBD, to feel that their voices are heard, and to truly feel how integral they are to the family.

Recommendations for Siblings

- **Afford siblings a voice:** Create opportunities for siblings to meet others in the same situation to talk about common experiences, and to talk with family members
- **Create sibling time:** Simple time alone with siblings, valuing their time and your time with them. Examples could include going for ice cream, or even food shopping
- **Reassure siblings:** Siblings need to know and hear explicitly how much they are loved. It may not seem necessary, but it becomes very important when siblings see the attention that is given to their siblings with DC/TBD.
- **Educate siblings:** Help minimize confusion through education and communication.

- **Create communication:** Keep communication ongoing during complicated medical times, and in times of forced separation. Use social media, Zoom, FaceTime, texting, calling, writing (letters or leaving notes), journal entries (sharing a journal back and forth between parent and child, writing entries to each other creating a private, yet regular form of communication), etc.
- **Honor relationships:** Sibling relationships are unique and play a role in defining the identity of each child. Avoid asking the sibling to carry roles of caregiving outside of age-appropriate tasks.
- **Allow for emotions and their expression:** Fear, loneliness, neglect, jealousy, worry, depression, pride, independence, etc.

Ongoing Impact

> *This, too, is life. Gently try not to devalue the life you have by longing for another life.*
>
> — Adult with DC

In the context of a rare, progressive illness, there is often the concern about what will happen next. It can be difficult to live in the present when worrying about the future. Growing up with DC/TBD, you will inevitably be exposed to someone who dies as a complication of the illness, either in the greater DC community or in your own family. As the DC/TBD community becomes more cohesive and individuals become more connected, they and their families offer each other tremendous support, information, and hope. At the same time, additional exposure to loss and grief emerges. Creating rituals to honor the life of someone who dies from the same illness you have honors not only the individual, but the entire community.

Questions from Adults with DC/TBD

- What will the future bring?

- How can I plan?
- What types of careers can I pursue?
- How do I negotiate DC/TBD and a relationship?
- If I want a family, how should I proceed?
- How would I manage my DC and manage a family?
- How will I integrate my DC/TBD into my life, but not have it stop me from doing things I want to do?
- How do I live with the uncertainty of the future?
- What if my symptoms cannot be controlled?

Family Matters

> *We cannot thank you enough from the depth of a mother's heart — desperately trying to save her children and their daddy.*
>
> —Parent of children with DC

The diagnosis of DC/TBD has a strong impact on the family system. Unlike other illnesses, at times the diagnosis of one family member may lead to the diagnosis of another. At certain points families may find themselves making decisions about experimental procedures and protocols that have been utilized with very few patients. These and many other factors create a unique experience of vulnerability, as individuals are forced to recognize the rarity of DC/TBD and the frailty of life.

Should an individual with DC/TBD deteriorate and alternate treatment options be considered, the family may again be thrown into an emotional crisis. One answer to such an experience can come in the context of support from the Team Telomere community. Being informed, empowered, prepared to take appropriate action, and feeling supported are all critical components, strengthening and supporting the resilience of persons with DC/TBD and their families. The support and assistance of

this community can mitigate against the stress and loneliness of this experience and can help prevent a sense of being immobilized, helpless, or hopeless.

The medical course of DC/TBD and its treatment continues to evolve, allowing for the emotional and physical sequelae also to continue to evolve. At every point there is a balance, endeavoring to prepare for the future and potential next steps, while actively living in the present.

Patient-Caregiver-Physician Relationships

Relationships with physicians are of tremendous significance to families affected by DC/TBD. Finding a physician who has expertise in DC, or is willing to work in collaboration with such a specialist is critical. The quality of these relationships often influences the patient and family's entire experience of DC/TBD and their quality of life. Helping navigate the course of the illness, and thinking through decisions, can help those facing DC/TBD feel much less isolated and much more in control.

Individuals and their family members must truly strive to become experts about DC/TBD and its treatment. Engaging with researchers and physicians who have devoted significant portions of their professional lives in the pursuit of knowledge about DC/TBD improves and inspires the lives of those facing the illness. This connection among patients, researchers, and clinicians creates a hopeful paradigm, which is truly best medicine for patients and their families.

Guidelines for Patients, Caregivers, and Physicians

- An initial psychosocial assessment of an individual diagnosed with DC/TBD (and parents if the person is a child) can serve as a helpful tool.
- Referrals to appropriate counseling and other resources (e.g., Team Telomere, individual counseling, support groups).

- - Encourage a dialogue among children or adults with DC/TBD or other bone marrow failure diseases (or other rare life-threatening illnesses) to minimize isolation and enhance self-esteem.
  - Encourage support group attendance for patients, parents, and siblings.
- Present information that is developmentally appropriate for individuals to enhance their understanding of and comfort with the diagnosis of DC/TBD.
- Encourage involvement with Team Telomere to help families develop and maintain an up-to-date knowledge base, to gain support, and to afford families an active role in supporting research. Encourage families to utilize Team Telomere resources:
  - Websites/webinars
  - Scientific sessions
  - Family meetings/retreats
  - Educational programs
  - Support of mentors
  - Group support
  - Team Telomere newsletters
  - Team Telomere and DC Facebook groups
  - Support from programs for persons with other rare illnesses, including other bone marrow failure and cancer predisposition syndromes
- Encourage families to create a working partnership with the physician/medical team.
- Encourage individuals with DC/TBD to become responsible and proactive with regard to their illness and medical care.
- Encourage adolescents and young adults to pursue their (academic, work, social) goals and dreams to prepare them for the transition to adulthood.
  - Help establish obtainable goals, with a next goal ever present.
- Encourage patients/family members to learn and stay abreast of salient treatment options.
- Encourage prevention and proactivity as they relate to illness manifestations.

- Work to make decisions with, and not for, families.
- Help the patient and family members to imagine the potential next illness manifestations.
- Help families adjust to living each day and focusing on activities apart from the illness as crucial components of day-to-day coping.
- Facilitate relationships and communication with DC/TBD professionals.
- Seek out specialists at times of decision-making.
- Establish relationships with counselors so they will be available at times of crisis.
- Join the Telomere Biology Disorder patient registry through RARE-X: https://teamtelomere.org/resources/rare-x/. Patient registries for rare diseases can be used to recruit patients for clinical trials, develop therapeutics, and better understand the patient population. Team Telomere is developing a secure patient registry to help identify DC/TBD patients around the globe and support research scientists and physicians in advancing knowledge of telomere disorders. Participation is optional but encouraged and can be done anonymously.

In rare, complex illnesses, such as DC/TBD, meetings with the treatment team can serve to educate and inform family members, garnering both practical and emotional support for the individuals facing DC/TBD. There is value in an ongoing dialogue with extended family members to discuss what is happening medically, and to create mutual support, as living with DC/TBD is truly a family endeavor.

Holding a broader family meeting (nuclear family members, and at times extended family members) with medical staff affords an opportunity for a wide range of issues and questions to be explored. Such meetings will exponentially increase family members' knowledge about DC/TBD, potentially increasing support that family members are able to provide to one another.

Traveling for Treatment

Because of the rare nature of DC/TBD, individuals often have to travel for evaluation and treatment with DC/TBD specialists in distant locations. Such medical travel is disruptive, challenging to organize, and can have enormous financial impact. Guidance from DC/TBD individuals and advocates who have traveled for treatment includes:

- **Researching and identifying centers:** Team Telomere maintains a network of physicians and clinicians at centers of excellence around the world who have specific expertise in DC/TBD, including genetic counselors, social workers, and specialists in organ and bone marrow transplantation in DC/TBD patients. Do not be afraid to ask your current medical care provider for referrals to other medical care providers. Several websites such as UNOS.org and bethematch.org provide statistics about the number and type of or solid organ and bone marrow transplants being done at centers around the world. Refer to Chapter 30, Finding Clinical Trials for more information about researching clinical trials.
- **Contacting centers:** Although it can seem daunting at first, do not be afraid to reach out directly or via your medical care provider to any center in the world where you think your loved one might find treatment or support. Keep emailing and calling as necessary, and do not get discouraged. Be friendly, patient, and persistent. Ask about the option of a virtual or online consultation to minimize travel when possible.
- **Creating a resume:** As DC/TBD individuals go through their medical journey, they may accumulate hundreds of pages of test results and medical notes that can become challenging for even the most dedicated medical care providers to wade through. When communicating with new medical care providers, it can be helpful to summarize the key points of the patient into a brief "resume" or patient profile that can be shared in PDF or printed format. Key information could include: patient name, photo (head shot), contact information, gender, age, height and weight, blood type, known allergies, summary of current physical condition,

family status, medical history in bullet points, relevant family history, description of treatments to date, list of centers and physicians (with contact information) where care has been provided, most recent lab results, and treatment goal. Be sure to include a link to the Team Telomere Clinical Guidelines! The 5-page health summary templates in the Appendix of Chapter 26, Transitioning from Pediatric to Adult Medical Care, can be useful for this.

- **Talking to other families:** Through Team Telomere's family chats, Facebook groups, and other forums, reach out to other patients and their family members. Compare notes about your experiences.

- **Working with your health insurance provider:** Talk to your insurance provider about what is covered if you travel outside your local area. Some health insurance providers can connect you with a specialized caseworker who can help you navigate and manage the costs of organ or bone marrow transplantation.

- **Reaching out for help from your network:** While some members of the family are traveling to distant medical centers, other family members may be left at home to continue their lives. Reach out to your support network to find help for those remaining at home, for example, asking a grandparent, aunt/uncle, or trusted friend to come stay with children left at home while parents are traveling with another child. As much as possible, keep in regular touch with family members at home, e.g., through text messaging, Zoom, FaceTime, and other electronic communications.

- **Reaching out for help at the center:** Large medical centers around the world often provide support services for patients who are traveling long distances to those centers for care. The range of services varies widely from center to center but can include such offerings as reduced-price, long-term housing for patients and caregivers, meal vouchers, free shower access, travel to and from the local airport, and translation services for international patients.

Conclusion

DC/TBD remains a difficult diagnosis to deliver and a complicated illness to live with. However, the connections within an affected family may run deeper than those of their healthy peers, as their experiences teach them a great deal about life. Parents of and those with DC/TBD sometimes describe having a greater appreciation for the things they do with their children, learning how to experience each day to its fullest and enjoying and valuing life. Patients, partners, and parents talk about being stronger than they realized and their ability to endure. The resilience exhibited by many of those affected with DC/TBD and their family members is truly remarkable; it reflects their ability to cope with this unique illness day-to-day. Given the complexity of family dynamics, the genetic components of DC/TBD, and the various roles and responsibilities that exist for affected individuals and family members, the support of Team Telomere and the commitment of professionals around the world united in advancing research and the treatment of DC/TBD, must be recognized as major components in enhancing the emotional well-being of those living with DC/TBD.

It is important for individuals to have the chance to tell their DC story; sharing one's own narrative leads to a personal place of healing.

> *Every time we talk to another DC patient or family, we learn. Even if our story is not exactly the same – we can all learn from each other's experiences. It's one of the most important parts of our community. It helped me to hear similar stories and feelings, especially in the beginning days.*
>
> *This is our first time telling our story and everyone was able to understand and/or relate. I've never experienced that before. The value of telling our story is to bring awareness of this rare disease to help a new family who may be struggling. [It is about] empowering others, processing my emotions, forming connections… I love to tell our story. I find it empowering to be able to educate others and raise awareness… so others can understand or try to understand that we are in a battle with a disease that doesn't have a cure.*
>
> *Talk, ask questions, take one step, the rest will follow. You might wobble and fall, but someone here will pick you up and help you take another step again.*
>
> —Compiled advice from members of the TBD community

Acknowledgements

Chapter 30

Finding Clinical Trials

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Introduction

The rapid advances in the causes and management of telomere biology disorders (TBDs) provide increasing opportunities for individuals with TBDs to participate in clinical trials aimed at improving the lives of those affected by these complex disorders.

Please note: Information about clinical trials should always be used along with the advice of your health care team.

What is a Clinical Trial?

A clinical trial is a research study performed in people to evaluate a medical, surgical, or behavioral intervention.

There are two main study types:

- **Clinical Trials:** Participants receive specific interventions according to the research protocol. Interventions include medical products (such as drugs, devices, procedures, changes to participants' behavior [such as diet]). Clinical trials may compare a new medical approach to a standard one that is already available, to a placebo that contains no active ingredients, or to no intervention.
- **Observational Studies:** Investigators assess health outcomes in groups of participants according to a research plan or protocol. Participants may receive interventions (which can include medical products such as drugs or devices) or procedures as part of their routine medical care, but participants are not assigned to specific interventions by the investigator (as in a clinical trial).

Where Do I Look for Trials?

ClinicalTrials.gov contains a database of federally and privately supported clinical trials being conducted around the world. This searchable registry can be used to locate information regarding a trial's purpose, inclusion/exclusion criteria, study locations, study coordinator's contact information, etc.

How Do I Find Appropriate Trials?

Search Fields

- The **Condition or disease** field tells the database to find all the studies with the disease that was entered. (For example, "Dyskeratosis Congenita" or "Telomere Biology Disorder.")
- The **Other terms** field can be used for additional terms you would like to search, such as a specific National Clinical Trial (NCT) number (unique study identifier in the format "NCTXXXXXXXX"), drug name, an investigator's name, etc.

- The **Country** field can be used to limit one's search to a specific country. If the United States is selected, search fields for State, City, and Distance will appear.

Figure 1. Searching for a clinical trial at ClinicalTrials.gov.

Searches Using Operators: OR, NOT, and AND

Words such as OR, NOT, and AND (in uppercase), are known as search operators. These words can be used in the search function to broaden or narrow a search. For example:

- Use OR to find studies that contain any of the words connected by OR.

  Example: Dyskeratosis Congenita OR Telomere Biology Disorder

  This search finds study records containing either the words "Dyskeratosis Congenita" or "Telomere Biology Disorder." Using OR broadens your search.

- Use NOT to find study records that do not contain the word following NOT.

Example: inherited NOT acquired

This search finds study records containing the word "inherited" but excludes records containing the word "acquired" from the search results. Using NOT narrows your search.

- AND is not necessary because the search function will automatically find study records that contain all the words specified in the search. However, AND can separate distinct concepts.

Additional Filters

Using the left sidebar, one can use the **Status**, **Eligibility Criteria**, **Study Type**, **Study Results**, **Study Phase**, **Funder Type**, and **Study Documents** filters.
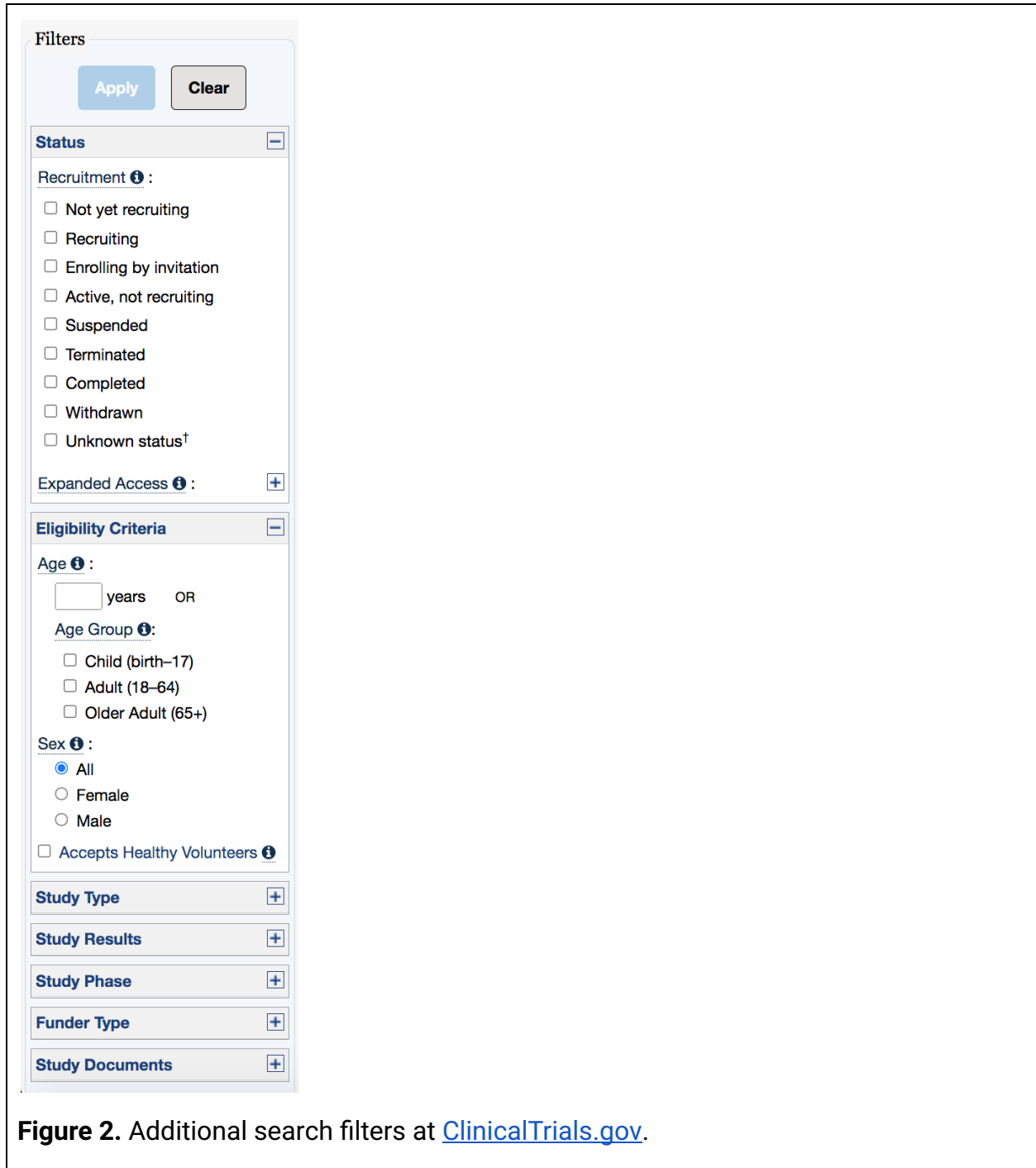
Figure 2. Additional search filters at ClinicalTrials.gov.

Recruitment Status

- **Not yet recruiting:** The study has not started recruiting participants.
- **Recruiting:** The study is currently recruiting participants.
- **Enrolling by invitation:** The study is selecting its participants from a population, or group of people, decided on by the researchers in advance. These studies are not open to everyone who meets the eligibility criteria - only people in that population, who are specifically invited to participate.
- **Active, not recruiting:** The study is ongoing, and participants are receiving an intervention or being examined, but potential participants are not currently being recruited or enrolled.
- **Suspended:** The study has stopped early but may start again.
- **Terminated:** The study has stopped early and will not start again. Participants are no longer being examined or treated.
- **Completed:** The study has ended normally, and participants are no longer being examined or treated.
- **Withdrawn:** The study stopped before enrolling its first participant.
- **Unknown:** A study's status was recruiting; not yet recruiting; or active, not recruiting but has passed its completion date. The status has not been verified within the past 2 years.

Eligibility Criteria

Eligibility criteria is the key requirements that people who want to participate in a clinical study must meet. Eligibility criteria consist of inclusion criteria (which are required for a person to participate in the study) and exclusion criteria (which prevent a person from participating).

Eligibility criteria can include whether a study accepts healthy volunteers, has age requirements, or is limited by biological sex. Additional information regarding eligibility criteria is often found under the details of a study.

Clinical Trial Phases

Clinical trials used in drug development are sometimes described by phase. These phases are defined by the Food and Drug Administration (FDA).

- **Preclinical:** Preclinical studies are sometimes called laboratory studies and can include studies on cell lines and animals. While pre-clinical studies give a lot of useful information, humans may differ in the way that the drug or treatment is absorbed, processed, and excreted. After the pre-clinical studies are completed and if the treatment still seems promising, the Food and Drug Administration (FDA) must give permission before the treatment can be tested in humans.
- **Phase I:** Describe clinical trials that focus on the safety of a drug. The goal is to determine the drug's most frequent and serious adverse events and how the drug is broken down and excreted by the body. These trials usually involve a small number of participants and healthy volunteers.
- **Phase II:** Gather preliminary data on whether a drug works in people who have a certain condition/disease. The drug may be compared to similar participants receiving a placebo (inactive substance) or a different drug. Safety continues to be evaluated; short-term adverse events and effectiveness are studied.
- **Phase III:** Gather more information about a drug's safety and effectiveness by studying different populations and dosages. The drug may be used in combination with other drugs. These studies typically involve more participants.
- **Phase IV:** Occurs after the FDA has approved a drug for marketing. These trials gather additional information about a drug's safety, efficacy, or optimal use.

In certain circumstances, phases may necessitate combining (e.g. Phase I/II), such as when trial on healthy participants is not possible.

Figure 3. Clinical trial phases infographic. (Figure adapted from hepb.org.)
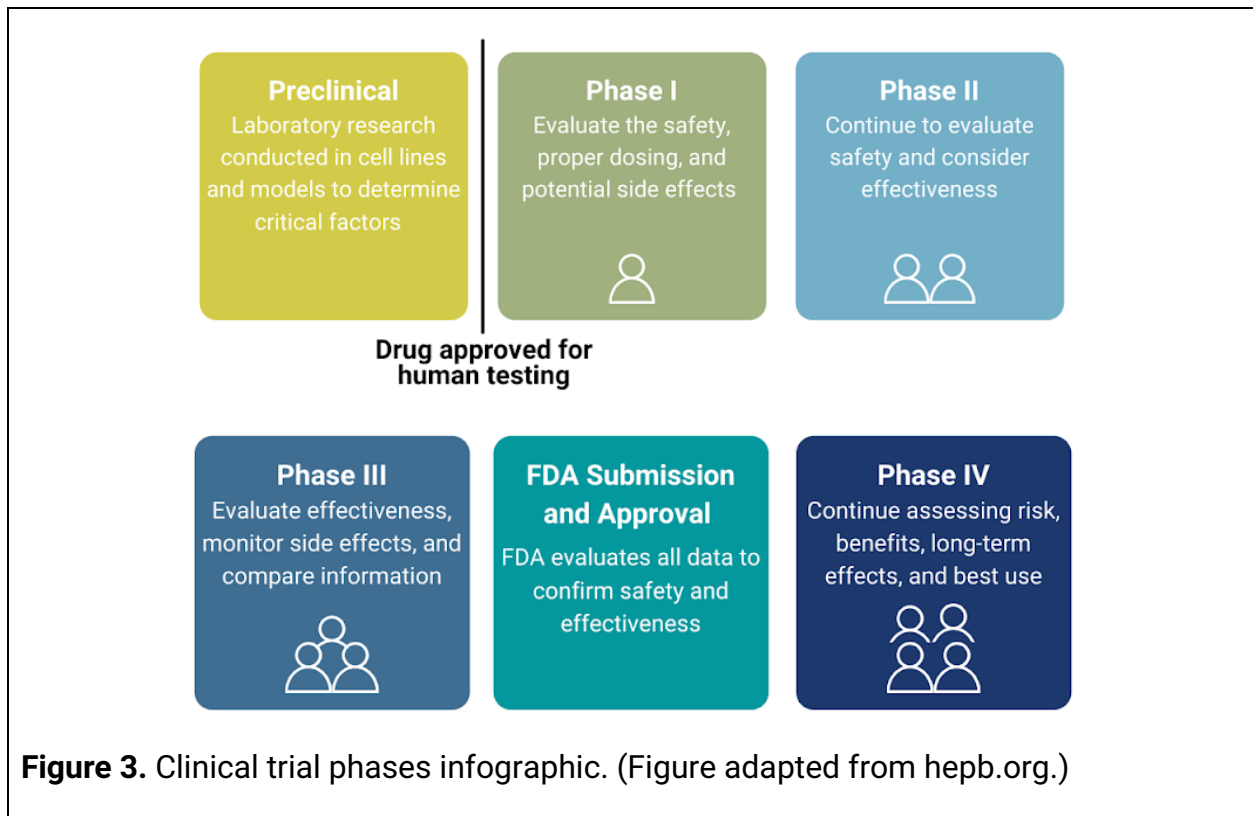
How are Study Participants Protected?

Informed Consent

Informed consent is a process used by researchers to provide potential and enrolled participants with information about a clinical study. This information helps people decide whether they want to enroll or continue to participate in the study. The informed consent process is intended to protect participants and should provide enough information for a person to understand the risks, potential benefits, and alternatives to the study. In general, a person must sign an informed consent document before joining a study to show that he or she was given information on the risks, potential benefits, and alternatives and that he or she understands it. Signing the document and providing consent is not a contract. Participants may withdraw from a study at any time - even if the study is not over.

Institutional Review Boards (IRB)

Every study must be reviewed, approved, and monitored by an institutional review board (IRB). An IRB is made up of doctors, researchers, and members of the community. Its role is to make sure that the study is ethical and that the rights of participants are protected. This includes making sure that research risks are minimized and are reasonable in relation to any potential benefits (among other responsibilities).

Future Implications

By participating in a clinical study, one contributes to medical knowledge. Much of what is known today about TBDs has been derived from clinical trials. The results of studies can make a difference in the care of future individuals with TBDs by providing information about the benefits and risks of diagnostic, preventative, therapeutic products, or interventions.

Questions to Consider Before Participating in a Trial

- What exactly is being studied?
- Why do researchers believe the intervention being tested might be effective? Why might it not be effective?
- Has it been tested before? What were the results from those tests?
- What are the possible interventions that I might receive during the trial?
- How will it be determined which intervention(s) I receive (for example, by chance)?
- Who will know which intervention I receive during the trial? Will I know? Will members of the research team know?
- How do the possible risks, side effects, and benefits of this trial compare with those of my current treatment?
- What will I have to do?

- What tests and procedures are involved?

- How often will I have to visit the hospital or clinic?

- Will hospitalization be required?

- How long will the study last?

- Who will pay for my participation?

- Will I be reimbursed for other expenses?

- What type of long-term follow-up care is part of this trial?

- If I benefit from the intervention, will I be allowed to continue receiving it after the trial ends?

- Will the results of the study be provided to me?

- Who will oversee my medical care while I am participating in the trial?

- What are my options if I am injured during the study?

Additional Resources

Additional clinical trials and information may be present under Team Telomere's Resources tab: https://teamtelomere.org/resources.

For information on the National Institutes of Health Inherited Bone Marrow Failures Syndromes study, please go to http://marrowfailure.cancer.gov/index.html.

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Chapter 31

What's on the Horizon: Research and Other Prospects

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Introduction

From coping with a rare disease diagnosis to navigating medical management and treatment, individuals with Telomere Biology Disorders (TBDs) and their family members may feel that they are facing a constant uphill battle. However, hope can be found knowing the advancements that have been made and that are on the horizon in the field of TBDs.

Science is moving faster than ever before, and medical knowledge has been expanding exponentially. It was estimated that the time it took to double medical knowledge in

1950 was 50 years. In 2020, medical knowledge was estimated to double in just 0.2 years – a mere 73 days [1].

This rapid progression of science is clearly evident in the field of TBDs. From when classic Dyskeratosis Congenita (DC) was first described in its most primitive form in the early 1900s to the present, countless advancements and discoveries have been made in the field of Telomere Biology Disorders: the elucidation of the underlying defects in telomere maintenance, the development of CLIA-certified flow FISH telomere length testing, the identification of TBD-related genes, the improved treatment of TBD symptoms and presentations, the initiation of TBD-specific clinical trials, and much more.

Essential to the field has been a better understanding of the range and spectrum of how TBDs present with symptoms in different individuals. Even at the start of the 21st century, TBDs were largely viewed as a pediatric bone marrow failure syndrome. However, it is now understood that individuals impacted by TBDs present with a range of physical presentations, with some individuals having no apparent physical symptoms of the disease and others displaying complex and serious problems in multiple parts of the body.

Around the globe, numerous clinicians, researchers, health professionals, and advocates are working towards the same goal: to better diagnose, manage, and treat TBDs.

Ultimately, each discovery and advancement is a piece of the puzzle. As the pieces come together, the future for people impacted by TBDs gets brighter.

Combining their perspectives, the authors contributing to this chapter have listed progress that individuals with TBDs, families, caregivers, clinicians, and scientists can expect in the coming years as our field continues to rapidly evolve.

For individuals, families, and caregivers impacted by TBDs, one can expect that

- The time to proper and accurate diagnosis will decrease
- The number of people diagnosed with TBDs will increase
- The track record for successful hematopoietic cell transplantation, lung transplantation, and liver transplantation at a wider number of medical institutions will improve
- Treatment options will increase and will be safer and more effective
- Improvements to treatments and continuing scientific advancements will not only lengthen lives but also increase quality of life
- TBD-specific prospective trials will increase; ongoing studies and patient registries will shine light on long term outcomes and disease progression
- Clinical trials evaluating novel therapeutic options that prevent end organ failure, supersede organ transplantation, and aim to target multiple body systems may begin
- Physicians and other health professionals will have increasing awareness of TBDs, leading to more clinical centers offering the specialized and multidisciplinary management and care that individuals with TBDs require
- Centers of Excellence will be developed, offering comprehensive multidisciplinary clinical management and care options to affected individuals and their families
- Access to community outreach will continue to increase, providing you education and guidance so you can become your (or your loved one's) best advocate and maintaining that *you are never alone*
- Awareness of how mental health impacts you and your family in the journey will increase
- Research will continue to be funded, and we will keep community needs at the forefront of clinical trials

For clinicians caring for individuals with TBDs, one can expect that

- TBD-specific experience across clinical disciplines and age ranges will continue to grow and appear in the published literature for reference
- Colleagues with specific expertise in TBDs will be increasingly identifiable and accessible for consultation and team care
- TBD-focused guidelines and clinical studies will define optimal preventive and interventional practices
- More children with TBDs will survive into adulthood, and more families with multiple generations at risk will be identified, requiring collaboration of adult and pediatric practitioners for transitional care and cross-referral

For scientists working to advance TBD science, one can expect that

- TBD-related genes and pathways will continue to be defined, yielding more opportunities to understand basic human telomere biology, and to define disease mechanisms and potential therapeutic targets
- TBDs will emerge as an ideal proving ground for the translation of cutting-edge scientific advances, including gene therapy, CRISPR/Cas9, and RNA medicine
- The forthcoming Team Telomere TBD research roadmaps and roundtables will identify critical unanswered questions in the field with the largest potential impact on patients and focus the research community's attention on developing effective treatments and cures
- Collaborative efforts between affected individuals, patient advocacy organizations, clinicians, and researchers – with patients and families at the heart of the discussions and work – will lead to exponential progress in basic and translational research

Team Telomere's vision is to see a world where every person impacted by Telomere Biology Disorders – including the affected individuals, caregivers, researchers, and clinicians – has accessible care, community, and resources, with the goal of positively

changing the course of this disease, driving toward improved treatments and ultimately one day a cure.

To see this vision to fulfillment, our mission is to provide information and support services to families worldwide affected by Telomere Biology Disorders, including Dyskeratosis Congenita, to encourage the medical community's research in finding causes and effective treatments, and to facilitate improved diagnosis by educating medical providers.

Our wholehearted commitment is to our community. We commit to working to find the path to accessible diagnosis and treatment. We commit to learning how to diversify science to be inclusive of all. We commit to working shoulder to shoulder with every stakeholder until a cure can not only be found but also become accessible to everyone affected by Telomere Biology Disorders. Our wish is that this promise brings hope to you and yours.

References

1. Densen P. Challenges and opportunities facing medical education. *Trans Am Clin Climatol Assoc*. 2011;122:48-58.

Glossary

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<Sharp brackets> mean "also known as."

Absolute Neutrophil Count (ANC): A measure of the number of neutrophils in the blood. Neutrophils are a type of white blood cell that help the body fight infection.

Acute Myeloid Leukemia (AML): A quickly progressive cancer of the myeloid line of blood cells, characterized by the rapid growth of immature white blood cells (also called myeloblasts) that accumulate in the bone marrow and interfere with the production of normal blood cells.

Adenoma: A benign tumor of a glandular structure or of glandular origin.

Adermatoglyphia: The absence of ridges on the skin on the pads of the fingers and toes, as well as on the palms of the hands and soles of the feet.

Adnexa: The appendages, or associated anatomical parts, of an organ.

Alanine Aminotransferase (ALT): An enzyme found in highest amounts in the liver. Injury to the liver results in release of ALT into the blood.

Alkylating Agent: A substance with mutagenic activity that inhibits cell division and growth and is used to treat some cancers.

Allele: A copy or alternate format of a gene.

Allogeneic: Involving, derived from, or being individuals of the same species that are sufficiently unlike genetically to interact antigenically.

Alopecia: Loss of hair.

Androgens: A synthesized, male sex hormone (testosterone).

Anemia: A condition in which the blood is deficient in red blood cells, in hemoglobin, or in total volume.

Anonychia: Congenital absence of the nails.

Antibody: Any of a large number of proteins produced normally by specialized B cells after stimulation by an antigen and act specifically against the antigen in an immune response. Some are produced abnormally by some cancer cells, and typically consist of four subunits including two heavy chains and two light chains. <immunoglobulin>

Antigen: Any substance foreign to the body that evokes an immune response either alone or after forming a complex with a larger molecule (as a protein) and that is capable of binding with a product (as an antibody or T cell) of the immune response.

Aplastic Anemia: A condition in which the bone marrow does not make enough blood cells.

Apoptosis: A genetically determined process of cell self-destruction that is marked by the fragmentation of nuclear DNA. It is activated either by the presence of a stimulus or by the removal of a stimulus or suppressing agent and is a normal physiological process eliminating DNA-damaged, superfluous, or unwanted cells. When halted (as by genetic mutation), it may result in uncontrolled cell growth and tumor formation. <programmed cell death>

Arteriovenous Malformations: Vascular anomaly characterized by abnormal connections between the arteries and the veins.

Ascites: Abnormal accumulation of serous fluid in the spaces between tissues and organs in the cavity of the abdomen. <hydroperitoneum>

Assisted reproductive technologies (ART): A broad term used to describe the range of technology used to assist human reproduction in the treatment of infertility.

Aspartate Aminotransferase (AST): An enzyme found in highest amounts in the liver. Injury to the liver results in release of AST into the blood. <aspartate transaminase>, <glutamic-oxaloacetic transaminase>

Ataxia: The inability to coordinate voluntary muscular movements.

Autosomal Dominant (AD): A trait or disorder that requires only one copy of the genetic mutation at a particular locus in order to express observable phenotype.

Autosomal Recessive (AR): Describes a trait or disorder that occurs when one variant is present on both alleles (copies) of a given gene in order to express observable phenotype.

Avascular Necrosis: Death of bone tissue due to impaired or disrupted blood supply (as that caused by traumatic injury or disease) and marked by severe pain in the affected region and by weakened bone that may flatten and collapse. <osteonecrosis>

B cells: A type of white blood cell that makes antibodies; are part of the immune system and develop from stem cells in the bone marrow. <B lymphocyte>

Biallelic Mutations: Mutations that occur on both copies of a single gene.

Blepharitis: Inflammation of the eyelids and especially of their margins.

Bone Marrow: The soft tissue filling the cavities of bones. Bone marrow exists in two types, yellow and red. Yellow marrow is found in the large cavities of large bones and consists mostly of fat cells and a few primitive blood cells. Red marrow is a hematopoietic tissue and is the site of production of erythrocytes and granular leukocytes. Bone marrow is made up of a framework of connective tissue containing branching fibers with the frame being filled with marrow cells.

Buccal Mucosa / Oral Mucosa: The inner lining of the cheeks.

Bullae: Large blisters on the skin that are filled with clear fluid.

Carcinogen: Any substance that causes cancer.

Carcinoma: A malignant tumor of epithelial origin

Carcinoma *in situ* (CIS): Carcinoma in the stage of development when the cancer cells are still within their site of origin (as the mouth or uterine cervix).

Carrier: In classical genetics, a carrier refers to an individual who carries one deleterious allele for an autosomal recessive disorder. In clinical discussions, a carrier may refer to an individual who carries a deleterious allele that predisposes to disease.

Cerebellar Hypoplasia: A neurological condition in which the cerebellum is smaller than usual or not completely developed.

Chemotherapy: The use of chemical agents in the treatment or control of disease. Especially the administration of treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing.

Cholestasis: Any condition in which the flow of bile from the liver is slow or blocked.

Clinical Trial: A type of research study that tests how well new medical approaches work in people. These studies test new methods of screening, prevention, diagnosis, or treatment of a disease. <clinical study>

ClinicalTrials.Gov: An online registry of clinical trials that are being conducted around the world. ClinicalTrials.gov is operated by the National Library of Medicine at the National Institutes of Health and can be accessed by anyone who has access to the internet.

Clubbing: Changes in the areas under and around the toenails and fingernails that may include the softening of the nail bed, forming of a sharper angle with the cuticle, bulging of the last part of the finger, and curving downward of the nail. <Digital Clubbing>

Cirrhosis: Widespread disruption of normal liver structure by fibrosis and the formation of regenerative nodules that is caused by any of various chronic progressive conditions affecting the liver.

Coats Retinopathy: The disorder causes blood vessels in the retina to be abnormally enlarged (dilated) and twisted. The abnormal vessels leak fluid, which can eventually cause the layers of the retina to separate (retinal detachment). These eye abnormalities often result in vision loss.

Common Variable Immunodeficiency (CVID): A disorder characterized by low levels of immunoglobulin (antibodies) and an increased risk of infection.

Comorbidity: A medical condition that exists simultaneously with and usually independently of another medical condition.

Complete Blood Count (CBC): A measure of the number of red blood cells, white blood cells, and platelets in the blood.

Compound Heterozygous: Usually refers to autosomal recessive disorders where an individual has two different abnormal alleles at a particular locus, one of each chromosome of a pair.

Conditioning Regimen: The treatments used to prepare a patient for stem cell transplantation that help make room in the patient's bone marrow for new blood stem cells to grow, prevent the patient's body from rejecting the transplanted cells, and help kill any cancer cells that are in the body.

Conjunctival Fornix: The loose arching folds connecting the conjunctival membrane lining the inside of the eyelid with the conjunctival membrane covering the eyeball.

Contraception: The deliberate prevention of conception.

Corpus Callosum: The band of commissural fibers uniting the cerebral hemispheres.

Corticosteroids: Any steroid hormone made in the adrenal cortex or in a laboratory setting; used medically as hormone replacement, to suppress the immune system, and to treat some side effects of cancer and its treatment.

Cutaneous: Relating to the skin.

Cytopenias: Deficiency of the cellular elements of the blood.

Cytotoxic: Toxic to cells.

Danazol: A synthetic sex hormone used in some studies of attenuation of accelerated telomere attrition.

De novo Variant: An alteration in a gene that is present for the first time in one family member as a result of a mutation in a germ cell (egg or sperm) of one of the parents or in the fertilized egg itself. <*de novo* mutation>

Diffusing Capacity of the Lungs for Carbon Monoxide (DL$_{co}$): A measurement to assess the lungs' ability to transfer gas from inspired air to the bloodstream.

Dilation: The act or action of stretching, widening, or enlarging a part of the body.

Deoxyribonucleic acid (DNA): The molecule inside cells that contains the genetic information responsible for the development and function of an organism. DNA molecules allow this information to be passed from one generation to the next. DNA is made up of a double-stranded helix held together by weak hydrogen bonds between purine-pyrimidine nucleotide base pairs: adenine (A) paired with thymine (T), and guanine (G) paired with cytosine (C).

Dual energy X-ray absorptiometry (DEXA or DXA): A procedure that measures the amount of calcium and other minerals in a bone by passing x-rays with two different energy levels through the bone; shows the strength and thickness of a bone.

Dyskeratosis Congenita (DC): A rare condition classified under a broad spectrum of genetic disorders known as telomere diseases. These diseases can often cause bone marrow failure and lung disease. People with DC frequently develop unusual skin pigmentation patterns, nail discoloration, white patches in the mouth (oral leukoplakia) and are especially susceptible to conditions that impair bone marrow function. DC may also cause pulmonary fibrosis, a condition that leads to the accumulation of scar tissue in the lungs, decreasing the flow of oxygen into the bloodstream. Although congenital (present at birth), the signs and symptoms of DC often may not appear until late childhood or early adolescence, and in some cases, not until adulthood.

Dyskerin: A protein involved in maintaining telomeres.

Dyslipidemia: A condition marked by abnormal concentrations of lipids or lipoproteins in the blood.

Dysphagia: Difficulty in swallowing.

Dysplasia: Cells that look abnormal under a microscope but are not cancer.

Dysplastic Nails: Ridging, flaking or poor growth of the nails.

Dyspnea: Difficult, painful breathing or shortness of breath.

Ectropion: The turning out of the eyelid so that the inner surface is exposed.

Edema: An abnormal excess accumulation of serous fluid in connective tissue or in a serous cavity.

Effusion: Escape of fluid from an anatomical vessel due to rupture.

Elastography: A type of imaging test that checks the liver for fibrosis. <liver elastography>

Endocrinologist: Physician treating problems associated with the endocrine system.

Engraftment: The process by which donor stem cells establish themselves successfully within the recipient.

Enterocolitis: Inflammation affecting both the small and large intestine.

Enteropathy: Disease of the intestinal tract.

Epigenetics: The study of heritable changes that do not affect the DNA sequence but influence gene expression.

Esophageal Stenosis: A narrowing of the esophagus that may interfere with swallowing.

Esophageal Web: Membranous structure in which a thin fold of tissue creates at least a partial obstruction of the esophageal lumen.

Esophagram: X-ray based test that takes pictures of the esophagus.

Exception points: A system to award increased waitlist priority to those patients whose severity of illness or risk of complications are not captured by the MELD score.

Excision: In a biopsy, the removal of the entire lump or suspicious area.

Exudative Retinopathy: A condition where blood vessels in the retina become abnormally enlarged and twisted. The abnormal vessels leak fluid, which can eventually cause the layers of the retina to separate (retinal detachment). This eye abnormality often results in vision loss.

Familial: Tending to occur in more members of a family than expected by chance alone.

Fibrosis: An increase of interstitial fibrous tissue.

FISH: A technique used to identify the presence of specific chromosomes or chromosomal regions through hybridization (attachment) of fluorescently-labeled DNA probes to denatured chromosomal DNA. Examination through a microscope under fluorescent lighting detects the presence of the colored hybridized signal (and hence presence of the chromosome material) or absence of the hybridized signal (and hence absence of the chromosome material). Also called fluorescence in situ hybridization.

Forced Vital Capacity (FVC): The amount of air that can be forcibly exhaled from the lungs after taking the deepest breath possible. This value is measured by spirometry, which is a common breathing test to check lung function.

Gene: The basic unit of heredity that occupies a specific location on a chromosome. Most genes code for a specific protein or segment of protein leading to a particular characteristic or function.

Genetic Anticipation: The phenomenon in genetic diseases where symptoms occur earlier and with increased severity in succeeding generations.

Germline: The cellular lineage of a sexually reproducing organism from which eggs and sperm are derived. The genetic material contained in this cellular lineage can be passed to the next generation.

Genotype: At its broadest level, genotype includes the entire genetic constitution of an individual. It is often applied more narrowly to the set of alleles present at one or more specific loci.

Graft Versus Host Disease (GVHD): A complication that can occur when T cells from a tissue or organ transplant, especially a bone marrow transplant, react immunologically against the recipient's antigens attacking cells and tissues.

Hematologic: Of or relating to blood or to hematology.

Hematopoiesis: The formation of blood or of blood cells in the living body.

Hematopoietic Growth Factor: Any of a group of glycoproteins that promote the proliferation and maturation of blood cells.

Hematopoietic Stem Cell Transplant (HCT or HSCT): The intravenous infusion of autologous or allogeneic stem cells collected from bone marrow, peripheral blood, or umbilical cord blood to reestablish hematopoietic function in patients whose bone marrow or immune system is damaged or defective.

Hemorrhage: A copious or heavy discharge of blood from the blood vessels.

Hepatic: Of, relating to, affecting, or associated with the liver. <hepatic injury> <hepatic insufficiency>

Hepatocellular: Of or involving hepatocytes. <hepatocellular carcinomas> <hepatocellular necrosis>

Hepatopulmonary syndrome: A condition caused by blood vessels in the lungs expanding (dilating) and increasing in number, making it difficult for red blood cells to properly absorb oxygen.

Heterozygous: The presence of two different alleles at a particular gene locus.

Hirsutism: Excessive growth of hair of normal or abnormal distribution.

Histology: The study of tissues and cells under a microscope.

Homozygous: The presence of two identical alleles at a particular gene locus.

Hoyeraal-Hreidarsson Syndrome (HH): A clinically severe Telomere Biology Disorder variant characterized by intellectual disability, intrauterine growth restriction, microcephaly, cerebellar hypoplasia, progressive combined immune deficiency, and/or bone marrow failure.

Human Papillomavirus (HPV): A sexually transmitted virus that is passed on through genital contact (such as vaginal and anal sex) as well as skin-to-skin contact.

Hyperhidrosis: Generalized or localized excessive sweating.

Hyperkeratosis: Excessive thickening of the outer layer of the skin.

Hypogammaglobulinemia: A deficiency of gamma globulins and especially antibodies in the blood.

Hypogonadism: Functional incompetence of the gonads especially in the male with subnormal or impaired production of hormones and germ cells.

Hypothyroidism: The clinical syndrome that results from deficient activity of the thyroid gland. It leads to lowered metabolic rate and general loss of vigor

Iatrogenic: Induced inadvertently by a physician or surgeon or by medical treatment or diagnostic procedures.

Idiopathic: Arising spontaneously or from an obscure or unknown cause.

Immunodeficiency: Inability to produce a normal complement of antibodies or immunologically sensitized T cells especially in response to specific antigens.

Immunoglobulin: A protein that is made by B cells and plasma cells (types of white blood cells) and helps the body fight infection. They are classified by structure and activity into five classes (immunoglobulin A; immunoglobulin D; immunoglobulin E; immunoglobulin G; immunoglobulin M).

Immunosuppression: Suppression (as by drugs) of natural immune responses.

In vitro: Outside the living body and in an artificial environment.

In vivo: In the living body of a plant or animal.

Incomplete Penetrance: Occurs when individuals who carry the pathogenic variant express the associated trait while others do not.

Inspiratory Rales: A fine, high-pitched crackling or rattling sound that can occur when someone inhales.

Intrauterine growth restriction: The failure of a fetus to attain its expected fetal growth at any gestational age.

Iron Chelation: Removal of iron from the blood through medication or phlebotomy.

Low-density Lipoprotein (LDL): A lipoprotein of blood plasma that is composed of a moderate proportion of protein with little triglyceride and a high proportion of cholesterol and that is associated with increased probability of developing atherosclerosis <bad cholesterol>, <beta-lipoprotein>, <low- density lipoprotein>

Leukemia: An acute or chronic blood cancer characterized by the type of white blood cell most prominently involved.

Leukocyte: A type of blood cell that is made in the bone marrow and found in the blood and lymph tissue. Leukocytes are part of the body's immune system. They help the body fight infection and other diseases. Types of leukocytes are granulocytes (neutrophils, eosinophils, and basophils), monocytes, and lymphocytes (T cells and B cells).

Leukoencephalopathy: Any of various diseases affecting the brain's white matter.

Leukoplakia: A white patch lesion found on a mucous membrane that cannot be scraped off. Leukoplakia is generally considered a precancerous condition, however its appearance may also result from a variety of hereditary diseases.

Luteinizing hormone (LH): A female hormone that, in combination with follicle stimulating hormone, stimulates the secretion of estrogen from ovarian follicles. In men, it is important in the development of interstitial tissue in the testis and for the secretion of testosterone <interstitial-cell stimulating hormone> <lutropin>

Lymphocyte: Any of the colorless weakly motile cells that originate from stem cells and differentiate in lymphoid tissue (as of the thymus or bone marrow), that are the typical cellular elements of lymph, that include the cellular mediators of immunity, and that constitute 20 to 30 percent of the white blood cells of normal human blood.

Lymphopenia: Reduction in the number of lymphocytes circulating in the blood of humans or animals.

Macrocytosis: The occurrence of larger-than-normal red blood cells.

Mammogram: An x-ray of the breast.

Menarche: The beginning of the menstrual function marked by the first menstrual period of an individual.

Menopause: The natural cessation of menstruation that usually occurs between the ages of 45 and 55. <climacteric>

Microcephaly: A condition of abnormal smallness of the head usually associated with intellectual delays.

Model for End-Stage Liver Disease (MELD) / Pediatric End Stage Liver Disease (PELD) Score: A scoring system to assess the severity of chronic liver disease.

Mosaicism: The occurrence of 2 or more cell lines with different genetic or chromosomal make-up, within a single individual or tissue.

Mucocutaneous Triad: Reticulated skin pigmentation, nail dystrophy, and oral leukoplakia.

Multifactorial: Caused or marked by a mode of inheritance dependent on a number of genes at different loci.

Myeloablative: The depletion of bone marrow cells, such as through the administration of chemotherapy and radiation therapy prior to a stem cell transplant.

Myelodysplastic Syndrome (MDS): A type of cancer in which the bone marrow does not make enough healthy blood cells (white blood cells, red blood cells, and platelets) and there are abnormal cells in the blood and/or bone marrow. Sometimes, myelodysplastic syndrome can transform into acute myeloid leukemia (AML).

Nail Dystrophy: This general term is used to describe changes in the shape, color, texture, and growth of the fingernails or toenails. The nails are often ridged, pitted, or abnormally curved.

Nasolacrimal Duct: The duct transmits tears from the lacrimal sac to the inferior meatus of the nose. It is also called the nasal duct.

Neoplasia: A process of tumor formation.

Neutropenia: Neutropenia is an abnormal decrease in the number of neutrophils, a type of white blood cells.

Neutrophil: A granulocyte that is the chief phagocytic white blood cell is called a neutrophil.

Next-Generation Sequencing (NGS) Technologies: This technology has been developed to speed up the process to sequence a human genome, DNA.

Nodular regenerative hyperplasia: A condition in which normal liver tissue transforms into multiple, small clusters (nodules) of replicating liver cells.

Ophthalmic: Structures that are in the region of the eye or that supply or drain the eye.

Opportunistic Infection: An infection caused by bacterial, viral or fungal pathogens that occurs more frequently and are more severe in individuals with weakened immune systems is said to be opportunistic.

Osteonecrosis: This disease is caused by reduced blood flow to the bones and joints. The lack of blood causes the bone to break down faster than the body can make enough new bone. The bone starts to die and may break down.

Osteopenia: This can cause bones to be weak and brittle, and increases the risk for broken bones. It is defined by a decrease in the amount of calcium and phosphorus in the bone. <bone loss>

Palliative Treatment: Care given to improve the quality of life and reduce pain. The goal of palliative care is to prevent or treat, as early as possible, disease symptoms and treatment side effects. It also attends to the psychological, social, and spiritual problems caused by the disease or its treatment and is often given with other treatments.

Pancytopenia: It is the reduction in the number of red and white blood cells as well as platelets. It results in fatigue due to the low numbers of red blood cells, frequent infections due to the low number of white blood cells, clotting problems due to the low number of platelets.

Pathogenic Variant: A genetic alteration that increases an individual's susceptibility or predisposition to a certain disease or disorder. When such a variant (or mutation) is inherited, development of symptoms is more likely, but not certain. <deleterious mutation> <disease-causing mutation> <predisposing mutation> <susceptibility gene mutation>

Patient registry: An organized system that uses observational study methods to collect uniform data (clinical and other) to evaluate specified outcomes for a population defined by a particular disease, condition, or exposure, and that serves a predetermined scientific, clinical, or policy purpose(s).

Parathyroid Hormone (PTH): The hormone of the parathyroid gland regulates the metabolism of calcium and phosphorus in the body. <parathormone.>

Phenotype: A phenotype is an individual's observable traits, such as height, eye color, and blood type. The genetic contribution to the phenotype is called the genotype.

Phimosis: Tightness or constriction of the orifice of the foreskin that prevents retraction of the foreskin over the glans.

Platelet: Are pieces of very large cells in the bone marrow called megakaryocytes. They help form blood clots to slow or stop bleeding and to help wounds heal. <thrombocyte>

Poikiloderma: Several disorders that are characterized by patchy discoloration of the skin.

Polypharmacy: The concurrent use of multiple medications by a patient to treat usually coexisting conditions and which may result in adverse drug interactions

Portal Hypertension: This type of hypertension in the hepatic portal system is caused by venous obstruction or occlusion that produces splenomegaly and ascites in its later stages.

Pulmonary Fibrosis: Pulmonary fibrosis is a condition in which the tissue deep in your lungs becomes damaged and scarred over time. This tissue becomes thick and stiff, making it difficult to breathe properly and preventing the blood from receiving adequate oxygen.

Pulmonary Function Test (PFT): Noninvasive tests that show how well the lungs are working. The tests measure lung volume, capacity, rates of flow, and gas exchange. This information can help diagnose and decide the treatment of certain lung disorders.

Purpura: This purplish or brownish red discoloration is caused by hemorrhage into the tissues. It is easily visible through the epidermis.

Rejection: An immune response in which foreign tissue (as of a skin graft or transplanted organ) is attacked by immune system components of the recipient organism.

Reticulated Skin Pigmentation: Skin pigmentation, or coloring, resembling a net. <hyperpigmentation>

Retinopathy: Any of various noninflammatory disorders of the retina including some that cause blindness.

Revesz Syndrome (RS): A variant of dyskeratosis congenita involving abnormalities in the light-sensitive tissue at the back of the eye (retina).

Schatzki's Rings: A local narrowing in the lower part of the esophagus that may cause dysphagia, or difficulty in swallowing.

Severe Combined Immunodeficiency (SCID): A rare congenital disorder of the immune system that is characterized by inability to produce a normal complement of antibodies and T cells and that usually results in early death.

Senescence: The process of growing old. In biology, senescence is a process by which a cell ages and permanently stops dividing but does not die.

Shelterin: A six protein complex known to protect chromosome ends and regulate telomerase activity.

Somatic Variant: An alteration in DNA that occurs after conception and is not present within the germline. Somatic variants can occur in any of the cells of the body except the germ cells (sperm and egg) and therefore are not passed on to children. Somatic variants can (but do not always) cause cancer or other diseases.

Squamous Cell Cancer (SCC): Cancer of a kind of epithelial cell. Is one of the major forms of skin cancer but can also occur in the digestive tract, lungs, and other areas of the body.

Strictures: Abnormal narrowing of bodily passages (as from inflammation, cancer, or the formation of scar tissue).

Syndrome: A group of signs and symptoms that occur together and characterize a particular abnormality or condition.

Taurodontism: A dental condition marked by the enlargement of the pulp cavities and the reduction of the roots.

Telangiectasias: A permanent dilation of preexisting blood vessels (capillaries, arterioles, venules) creating small focal red lesions, most commonly in the skin or mucous membranes.

Telomerase: A ribonucleoprotein (RNA) that is an enzyme that adds DNA sequence repeats to the end of DNA strands in the telomere regions, which are found at the end of chromosomes.

Telomere: The end of a chromosome. Telomeres are made of repetitive sequences of non-coding DNA that protect the chromosome from damage. Each time the cell divides, the telomeres become shorter. Eventually, the telomeres become so short that the cell can no longer divide and it dies.

Telomere Biology Disorder (TBD): Telomere biology disorders are a complex set of illnesses defined by the presence of very short telomeres.

Thelarche: The beginning of breast development at the onset of puberty.

Thrombocytopenia: Persistent decrease in the number of blood platelets.

Trichiasis: A turning inward of the eyelashes often causing irritation of the eyeball.

Urethral Stenosis: A narrowing of the diameter of the urethra.

Urogenital: Of, relating to, affecting, treating or being the organs or functions of excretion and reproduction.

Varices: An abnormally dilated or swollen blood or lymph vessel, especially a vein.

Veno-Occlusive Disease: Disorder in which veins are partially or completely obstructed or the blood flow through the veins is less than optimal.

X chromosome inactivation: Refers to the silencing of one X chromosome in female mammalian cells to equalize the gene products from the X chromosome between females and males. Skewed X-chromosome inactivation can occur when the X-inactivation of one X chromosome is favored over the other, leading to an uneven number of cells with each chromosome inactivated.

X-linked recessive (XLR): Refers to genetic conditions associated with pathogenic variants in genes on the X chromosome. A male carrying such a variant will be affected, because they carry only one X chromosome. A female carrying a variant in one gene, with a normal gene on the other X chromosome, is *generally* unaffected.

Resources

Clinical Telomere Length Testing

Clinically certified testing of telomere length by flow FISH is available in Canada, the USA, Switzerland, Germany, and Australia.

Flow FISH provides a measurement of average telomere length in cells of leukocyte (white blood cell) subsets and is the only test that is clinically available in certified labs and validated for the diagnosis of TBDs (see Chapter 3, Diagnosing Telomere Biology Disorders).

Telomere length testing laboratories certified through the Clinical Laboratories Improvement Amendments or CLIA, which govern testing on human subjects, are able to measure telomere lengths according to standards accepted by medical professionals.

Listed are facilities that offer CLIA-certified flow FISH telomere length testing:

Repeat Diagnostics Inc. / Geraldine Aubert, PhD
309-267 West Esplanade Ave.
North Vancouver, BC V7M 1A5
Phone: 604-985-2609
Fax: 778-340-1144
www.repeatdiagnostics.com

Johns Hopkins Genomics-MDL
1812 Ashland Ave
Room 245
Baltimore, MD 21205
Phone: 443-287-7291
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Additional Resources

Updated patient/caregiver and medical/scientific resources can be found through the resources tab on Team Telomere's website teamtelomere.org/resources or consulted through info@teamtelomere.org.

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