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Azra Raza, MD, Columbia University Medical Center
DNA is the chemical compound containing the instructions to develop and direct the activities of nearly all living organisms. The genome is the complete set of DNA in an organism. Researchers are using new techniques to explore the human genome and understand bone marrow failure diseases.

### Overview of MDS Genomics

**Luca Malcovati**, MD  
University of Pavia

Dr. Malcovati summarized the current understanding of genomics in MDS. Experts believe that MDS is the result of at least one somatic mutation (acquired after birth) in a hematopoietic stem cell (HSC). HSCs can turn into any type of blood cell in the bone marrow. HSCs with one of these mutations can make abnormal copies (clones) of themselves that can reproduce more easily than healthy cells. These abnormal clones take over much of the bone marrow, resulting in shortages of healthy blood cells.

In the last few years, researchers have identified several somatic mutations in many patients with MDS. At least 10% of patients with MDS have mutations in four to six genes (including SF3B1, TET2, SFRS2, and ASXL1). Changes in these genes tend to occur early in MDS development.

Some healthy adults start forming abnormal clones as a result of mutations in genes that are associated with aging even though they don’t have MDS. These mutations are very rare in people in their 50s. But up to 10% of those older than 70 and up to 20% of those older than 80 have these mutations. These mutations might trigger the expansion of the initial abnormal clone and development of MDS.

DNMT3A is mutated in many blood cancers, including MDS and acute myelogenous leukemia (AML). Patients with AML who are in complete remission after chemotherapy sometimes still form blood cells with mutant DNMT3A. These mutations likely play a role in driving the expansion of clones that lead to leukemia.

Somatic mutations in SF3B1, an RNA splicing gene, are common in patients with refractory anemia with ring sideroblasts, a type of MDS. Research shows that 98% of patients with an SF3B1 mutation develop ring sideroblasts. The mutation seems to help cause ring sideroblasts. SF3B1 mutations happen before other mutations in many patients with low-risk or intermediate-risk MDS.

Although DNMT3A mutations become more common as people age, SF3B1 is only present in people who are at least 70 years old. Therefore, aging of the bone marrow might lead to the expansion of MDS clones with these mutations.

### Overview of Aplastic Anemia Genomics

**Austin Kulasekararaj**, MBBS MD  
MRCP FRCPath  
King’s College London

Dr. Kulasekararaj wanted to find the mutations in genes related to MDS that are present in patients with aplastic anemia. The goal was to learn whether some patients with aplastic anemia are more likely to develop MDS.

The first study showed that ASXL1 mutations were the most common abnormal genes in patients with aplastic anemia, followed by mutations in DNMT3A and BCOR. In half the patients, less than 10% of bone marrow cells had the mutation. Patients who had had aplastic anemia for more than 6 months and had a somatic (not inherited) mutation in an MDS gene thereby were much more likely to develop MDS.

Some patients in the study had monosomy 7 (only one copy instead of two copies of chromosome 7) at the time their aplastic anemia progressed to MDS. These patients had different mutations months before their disease progressed from those whose disease didn’t progress.

Somatic mutations that drive the expansion of abnormal clones (copies) of blood cells in bone marrow are common in elderly people. These mutations are most frequent in DNMT3A, TET2, and ASXL1, and they can predict how long a patient will survive.
In a patient who had been diagnosed with paroxysmal nocturnal hemoglobinuria (PNH) 20 years earlier, clone size gradually decreased with eculizumab treatment and he went into remission. However, the disappearance of the PNH clone did not result in normal blood cells in his bone marrow. Rather, the patient formed abnormal clones as a result of other somatic mutations.

Some patients have mutated clones at the time of their aplastic anemia diagnosis. These clones can become bigger or they might disappear with or without treatment. The question is what these changes in clone size mean in the context of a disease that is influenced by the immune system.

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**Genomics of Aplastic Anemia**

**Neal Young, MD**
National Heart, Lung & Blood Institute, NIH

Dr. Young reviewed the latest findings on the genomics of aplastic anemia. The worst complication of aplastic anemia is that it can evolve to MDS and acute myelogenous leukemia (AML).

A recent study assessed mutations in 106 genes associated with blood cancers in 439 patients with severe aplastic anemia. A third of the patients had somatic (not inherited) mutations in genes (DNMT3A, ASXL1, BCOR, and BCORL1) that are common in patients with MDS, AML, or both. Mutations in two of these genes, DNMT3A and ASXL1, also become more common with aging in healthy people.

The study showed that only a few genes are mutated in aplastic anemia, whereas many genes are mutated in MDS. Patients with severe aplastic anemia tended to have these mutations in fewer bone marrow cells than patients with MDS or other blood cancers. The individual mutations did not predict length of survival or likelihood of progression to MDS or AML.

The behavior of clones, or abnormal copies of cells that form blood cells, over long periods of time in patients with aplastic anemia is striking. Some patients who have clones with ASXL1 or DNMT3A mutations respond well to immunosuppressive treatment and their clones remain stable for years. Clones with BCOR mutations tend to disappear after the first year. The mutations in a patient younger than 60 can predict response to treatment, but this is less obvious in patients older than 60.

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In one example, a 50-year-old male with aplastic anemia had a clone with an ASXL1 mutation. The patient did not want to have stem cell transplantation because he was doing well. He had immunosuppressive treatment instead, and his ASXL1 clone disappeared within 3 months, but the clone eventually came back. A reasonable way to manage this patient is to monitor him and if the clone gets worse or develops new mutations, he could undergo transplantation.

Healthy people can have clones with genetic mutations. These clones do not form healthy blood cells. When a patient has bone marrow failure, clones with mutations take over the bone marrow, replacing healthy cells.

Telomeres are the ends of chromosomes that help keep chromosomes stable. As people age, their telomeres get shorter. People with aplastic anemia tend to have very short telomeres as a result of mutations in genes, including TERT and TERC, that control telomeres. Danazol (Danocrine), an artificial steroid, elongates telomeres and increases counts of healthy blood cells in patients with aplastic anemia.

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**Experimental Mouse Breeds for Research on Genome Lesions**

**Rose Ann Padua, PhD**
University Paris-Diderot

Dr. Padua discussed new experimental mouse breeds and summarized some recent studies in these mice to assess new treatments.

Many new breeds of mice are now available for studies of changes in several genes that are associated with MDS, aplastic anemia, and/or acute myelogenous leukemia (AML) in people. These mice develop bone marrow failure and other features of these diseases in people, although the disease is often mild.

Dr. Padua has bred a mouse breed with abnormalities in one of two genes: NRAS and BCL-2. NRAS mutations are associated with MDS, aplastic anemia, and/or acute myelogenous leukemia (AML) in people. These mice develop bone marrow failure and other features of these diseases in people, although the disease is often mild.

Dr. Padua has bred a mouse breed with abnormalities in one of two genes: NRAS and BCL-2. NRAS mutations are associated with MDS, and increased BCL-2 expression is associated with AML. The mice develop a disease that resembles MDS or AML, depending on where BCL-2 is expressed. Expression in a primitive cell (also known as a stem cell) gives rise to MDS and drives expression to a more mature cell results in AML. Mice with MDS-like features live a bit longer than those with AML. These mice have a lot of DNA damage and bad repairs of DNA damage.
N-acetyl cysteine (NAC) is a form of L-cysteine, an amino acid (protein building block). Treatment with NAC can reverse the DNA damage and bad repairs in the experimental mice with mutated NRAS or BCL-2. However, studies have not yet replicated this result in patients.

ABT-737 is a drug that induces the cancer cell death by inhibiting the BCL-2 proteins that promote cancer cell survival. ABT-737 seems to target cells that initiate leukemia. Mice treated with ABT-737 live a lot longer than untreated mice.

pVAX14 is a vaccine containing DNA sequences that change the immune system. In mice with MDS, treatment with pVAX14 reduces the proportion of blasts (immature white blood cells that are abnormal) in bone marrow and eliminates MDS in some mice. Azacitidine is the standard treatment for MDS. Azacitidine prolongs survival in mice with MDS or AML after MDS.

The combination of azacitidine with pVAX14 and all-trans retinoic acid results in longer survival in mice with MDS, compared to azacitidine or no treatment. Dr. Padua believes that this combination increases the immune response in these mice. Dr. Padua is planning a Phase I/II clinical trial of the vaccine combined with azacitidine in patients with high-risk MDS. If patients tolerate the vaccine, they will also be treated with all-trans retinoic acid. Retinoic acid, a vitamin A derivative, on its own changes the immune system and cooperates with the DNA vaccine to increase survival of MDS mice. This approach has the potential to be applied to other cancers.

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**Aging and Blood-Cell Formation**

**Christopher Park** MD, PhD
Memorial Sloan Kettering Cancer Center

Dr. Park talked about the relationship between normal aging and MDS. Blood (hematopoietic) stem cells, or HSCs, can turn into all types of blood cells. But as people get older, HSCs gradually lose their ability to produce red cells and certain types of white blood cells (especially lymphocytes). In some older people, HSCs can acquire mutations that lead to a higher risk of developing MDS or acute myelogenous leukemia (AML).

The factors that produce these changes in aging HSCs are not completely understood. But some of the contributing factors include buildup of reactive oxygen species, which are molecules containing oxygen that can damage DNA. Other causes are increases in DNA damage, changes in the epigenome (alterations in gene activity not due to mutations in DNA), and buildup of damaged proteins. The number of normal stem cells drops, and the remaining stem cells can’t form mature blood cells. Some of these changes in aged stem cells can be reversed in experimental animals. Therefore, some of these strategies might be useful for treating MDS.

The HSCs of older people are similar in many ways to the HSCs of people with MDS. For example, the number of HSCs increases, and these HSCs lose their ability to form normal numbers of blood cells in both MDS patients and older adults. However, people with MDS have even greater reductions in the production of white and red blood cells and a higher risk of developing AML. The major reason why most patients with MDS do poorly is their inability to produce normal numbers of red and white blood cells.

Recent studies have shown that MDS HSCs are different from aging HSCs in several ways. For example, the two types of HSCs have different DNA methylation patterns (chemical changes in the DNA that stop genes from carrying out normal activities) and express different genes and proteins. MDS HSCs also have different genetic characteristics from aging HSCs, and they develop specific DNA mutations that cause disease.

Some of the gene signatures of MDS HSCs might be useful for predicting responses to treatment. For example, studies show that HSCs in patients with MDS who respond to decitabine (Dacogen) treatment are more like normal HSCs than HSCs in patients who do not respond to treatment. Therefore, it might be possible to develop better ways to determine whether patients will respond to therapy. This would help clinicians choose the right treatments when patients are diagnosed.

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**Targeting the Immune System to Treat MDS**

**Alan List** MD
H. Lee Moffitt Cancer Center

Dr. List discussed the role of inflammation and the immune response in MDS. He also explored the use of experimental treatments that interfere with inflammation to treat MDS.
Inflammation is the body’s normal response to protect itself from infections and injuries. When the body has an injury, immune system cells (including antibodies and different types of white blood cells) travel to the site in the body that needs help. After the cells fight the foreign invaders, the inflammation typically disappears. But inflammation sometimes persists and damages surrounding tissues. For this reason, the immune system and inflammation play a role in the development of many diseases, including MDS.

Chronic activation of the body’s immune system is involved in both the aging of HSCs and development of MDS. MDS HSCs have abnormally active immune receptors known as Toll-like receptors (TLRs) that help the immune system recognize and respond to foreign substances. This activation of TLRs leads to the expansion of abnormal MDS bone marrow stem cells (HSCs) and impairs formation of blood cells.

Myeloid-derived suppressor cells (MDSCs) are part of the immune system. Their numbers grow with age, infection, inflammation, and cancer. They lead the immune system to tolerate cancerous cells and dampen the formation of healthy blood cells. Unlike MDS clones (abnormal copies of cells), MDSCs don’t have abnormal chromosomes or gene mutations. MDSCs come from noncancerous HSCs and develop before MDS clones.

The S100A9 protein helps direct inflammation and the immune response. The amount of S100A9 increases with inflammation and aging in parallel with increases in the numbers of MDSCs. This protein promotes insulin resistance (which leads to diabetes) and atherosclerosis (fat buildup in artery walls). Levels of S100A9 are higher in the blood of patients with lower-risk MDS.

Several experimental treatments can neutralize S100A9 by binding to it or by stopping it from sending out signals. These compounds have shown potential in mouse studies to treat lower-risk MDS by suppressing the rapid death (by a process known as pyroptosis) of healthy cells and increasing counts of HSCs.

The NLRP3 inflammasome is a complex of proteins that play a key role in inflammation and pyroptosis. Compounds that neutralize this protein complex can also suppress pyroptosis of healthy cells, reduce MDSC counts and reactive oxygen species (molecules containing oxygen that can damage DNA), and restore effective blood cell formation.

The inherited bone marrow failure syndromes include Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome, and congenital amegakaryocytic thrombocytopenia. Patients with these syndromes have a higher risk of developing severe aplastic anemia and MDS. Many patients have certain features (such as physical abnormalities). But some patients have none of the classical signs and symptoms of the disease.

Patients with these syndromes often can’t tolerate the standard treatments used to suppress the immune system before stem cell transplantation. Also, treatments are most likely to cure the syndrome when it’s caught early. So diagnosing these inherited diseases accurately is important to help clinicians choose the right treatments.

Patients with inherited bone marrow failure syndromes often do very well with stem cell transplantation. But their doctors need to be aware that the patient has an inherited disorder so that they arrange testing for family members who are potential donors so that the donor doesn’t have the same inherited disease as the patient.

Most cases of AML and MDS develop after age 50 or so. So why do some young people develop MDS? One possibility is that they inherited a genetic mutation because people with some of these syndromes have a higher risk of MDS or AML at a young age. It’s also possible that bone marrow failure syndromes are biologically different in young patients and older patients.

When Dr. Shimamura screened 110 patients younger than 45 with MDS, she found mutations in bone marrow failure genes in 14% of the patients. Although each mutation is rare, having one of these mutations is not really rare.
Now that doctors can rapidly screen patients for many different genes at once in a cost-effective way, they need to rethink genetic screening for bone marrow failure diseases. A family history of the disease should trigger testing for an inherited disease. But lack of a family history or of physical signs should not prevent doctors from doing this type of screening. Dr. Shimamura recommended genetic testing for all children and young adults and for some older adults with bone marrow failure syndrome.

Dr. Shimamura recommended genetic testing for all children and young adults and for some older adults with bone marrow failure syndrome.

MDS and Leukemia in Fanconi Anemia

Jean Soulier, MD, PhD
Hôpital Saint-Louis

Dr. Soulier discussed the relationship between Fanconi anemia, MDS, and acute myelogenous leukemia (AML). He introduced the genetics of Fanconi anemia and potential treatments.

People who have Fanconi anemia have an inherited mutation in one of the 18 known Fanconi anemia genes. They have a very high risk of developing bone marrow failure during childhood and, later in life, a very high risk of MDS or AML.

Hematopoietic stem cells (HSCs) that turn into healthy blood cells in patients with Fanconi anemia build up DNA damage. As a result, the HSCs stop reproducing and then get old or die. So patients have very few HSCs when they are diagnosed with Fanconi anemia.

Patients with Fanconi anemia tend to get a specific type of MDS: refractory cytopenia with multilineage dysplasia or refractory anemia with excess blasts. They also develop the type of AML (secondary AML) that starts during or after MDS.

To find the genes that drive MDS and AML development in Fanconi anemia, Dr. Soulier studied samples from 50 patients who had Fanconi anemia and MDS or AML. He found some major abnormalities in these patients’ chromosomes. The most common abnormalities were duplication of the long (q) arm of chromosome 1 or of chromosome 3. These abnormalities probably let abnormal bone marrow cells reproduce more easily than healthy cells and eventually take over the bone marrow. In addition, many patients had an abnormality in the RUNX1 gene. In contrast, the other common MDS and AML genes were rarely mutated.

These results could be useful for finding treatment strategies for different phases of Fanconi anemia and MDS or AML. Patients can be grouped according to the extent bone marrow failure, abnormal bone marrow cells, and abnormal chromosomes. Recommended treatment for each group then ranges from yearly monitoring of the bone marrow for the mildest disease to HSC transplantation for the more severe stages.

Lengthening Telomeres to Treat Aplastic Anemia

Rodrigo Calado, MD, PhD
University of Sao Paulo

Dr. Calado described the role of telomeres, the ends of chromosomes, in aplastic anemia. He also talked about ways to elongate telomeres to treat aplastic anemia. Telomeres maintain the stability of chromosomes in cells. They become shorter with age. Eventually, even cells that express telomerase, an enzyme that maintains telomere length, can’t stop the shortening process. However, telomerase probably slows down the shortening process.

Several genes help control telomere maintenance and repair, including TERT and TERC. Mutations in these genes play a role in diseases (known as telomeropathies) that result from short telomeres, such as aplastic anemia, pulmonary fibrosis, and some types of liver disease. The telomeropathies all result from a mutation in a telomerase gene, but they have very different effects on patients.

Dr. Calado described a study of a brother and sister with short telomeres and mutations in TERT and TERC. The brother was healthy. But his telomeres were short and his bone marrow was packed with red blood cells, which can be a sign of MDS. The sister had short telomeres and a shortage of red blood cells in bone marrow. She needed regular red blood cell transfusions. Activation of TERT and TERC in cells from these siblings elongated telomeres. However, the cells still showed abnormal telomerase function and defective telomere elongation. Also, their immature blood cells still had limited ability to form healthy blood cells. The investigators then grew cells from these two siblings in low oxygen. The telomeres grew longer and TERT activity increased.
Sometimes, several members of a family have the same mutations, but some members have short telomeres and other don’t. In one study, family members with mutations in two genes, WRN and BLM, had very short telomeres. Family members without these mutations had normal telomeres. Abnormalities in other genes that are involved in DNA repair and might help maintain telomeres are probably involved in aplastic anemia.

Androgens (male sex hormones) and estrogens (female sex hormones) seem to increase telomerase activity. In a clinical trial of danazol (Danacrin®), an androgen, most patients with aplastic anemia had TERT or TERC mutations. The study ended early because the treatment was so effective in elongating telomeres. Danazol also increased red blood cell, white blood cell, and platelet counts. However, the treatment had side effects that some patients couldn’t tolerate.

Another study of nandrolone, an androgen that should have fewer side effects, is recruiting patients. Blood cell counts have increased in the nine patients in the study so far.

Lesson 2: GATA2 deficiency can be associated with Mycobacterium kansasii infection. This infection was responsible for 2.5% of cases of disseminated nontuberculous mycobacteria infections in people with AIDS and up to 7% of disseminated nontuberculous mycobacteria in those with hairy cell leukemia. Most people with these infections don’t get very sick, but the infections can cause serious diseases. Patients with GATA2 deficiency have a much higher risk of Mycobacterium kansasii infection, which increases their likelihood of forming abscesses in lungs and other organs. All of these abscesses can be treated.

Lesson 3: People with GATA2 deficiency can develop lung abnormalities. Over time, most patients with GATA2 deficiency develop pulmonary alveolar proteinosis, which is otherwise rare. The reasons why this happens have yet to be found.

Lesson 4: About 5% of patients with GATA2 deficiency have Clostridium difficile infection. The infection can be very severe and difficult to treat, including in children.

Lesson 5: Patients sometimes have signs of GATA2 deficiency but no GATA2 mutation. One patient, for example, had very low white blood cell counts and a fungal infection, but testing did not find a GATA2 mutation. However, she did have a mutation in ADA2 (CECR1). So she didn’t actually have GATA2 deficiency. This is an example of the GATA2 syndrome actually being due to a completely different disease that affects some of the same cells.

GATA2 Deficiency and Bone Marrow Failure

Steven M. Holland, MD
National Institute of Allergy and Infectious Diseases, NIH

Dr. Holland described five lessons learned about GATA2 deficiency. People with this genetic disease can develop severe infectious, breathing problems, and childhood cancer. Deficiency in the GATA2 gene can cause the following in children: low white blood cell counts, MDS, acute myelogenous leukemia (AML), acute lymphocytic leukemia, and chronic myelomonocytic leukemia.

Lesson 1: Patients with GATA2 deficiency sometimes have Epstein-Barr virus (EBV) infection in smooth muscle cells. Doctors used to think that only patients with HIV infection had EBV in their smooth muscle cells. Researchers have now defined a new disease in which EBV is out of control and sometimes contributes to cancer. Several genes are associated with EBV infections and cancers, and GATA2 belongs on this list.
PATHOPHYSIOLOGY AND NEW MOLECULAR TARGETS OF BONE MARROW FAILURE

Session Co-Chairs:
Benjamin Ebert, MD, PhD
Brigham and Women’s Hospital

Matthew Walter, MD
Washington University in St. Louis

Inherited Bone Marrow Failure Diseases

Lucy Godley, MD
University of Chicago

Dr. Godley discussed inherited bone marrow failure diseases, which are much more common than experts had thought, even in adults. These inherited cancer syndromes are contributing to the understanding of how cancer develops and the discovery of effective treatments.

MDS, AML, and chronic myelomonocytic leukemia begin in immature cells that form blood cells in bone marrow. Dr. Godley talked about three categories of inherited mutations associated with these bone-marrow failure diseases: those that are associated with bone-marrow cancers only, those associated with defective platelet function, and those that affect other organs.

Mutations Associated with Bone-Marrow Cancers Only

CEBPA is a gene with mutations associated with bone marrow cancers only. Patients with leukemia who have this mutation have a mutation in both alleles (copies) of CEBPA. In about 10% of patients, one of the mutations is inherited and the other is acquired after birth. For this reason, Dr. Godley recommends that doctors order genetic testing for all patients with CEBPA mutations in both alleles because all of them will develop leukemia.

About 57% of patients with one inherited and one acquired CEBPA mutation live for at least 10 years, whereas the 10-year survival rate for those with two acquired mutations is about 54%. Patients with CEBPA mutations often go into remission with chemotherapy and stay in remission for years. But they can have relapses several years later that are actually new primary cancers.

Mutations in DDX41 are also associated with bone marrow cancers. Families with inherited mutations in DDX41 tend to develop leukemia at age 65 years, on average. The age of leukemia diagnosis is typically older in previous generations than in younger generations within the same family.

Mutations Associated with Defective Platelet Function

Inherited RUNX1 mutations predispose people to platelet clumping, defective platelet function, and bone-marrow cancers. Patients with these inherited mutations who develop bone marrow cancers also have mutations that they acquire after birth. The lifetime risk of a bone marrow cancer in people who carry the RUNX1 mutation ranges from 20% to 65%.

The most common tests for genetic mutations in people with bone-marrow cancers don’t include ANKRD26 mutations. Inherited mutations in this gene increase a person’s risk of thrombocytopenia, or platelet shortage in bone marrow, as well as abnormal platelet function and leukemia. Inherited ETV6 mutations also increase risk of bone-marrow cancers that can start at almost any age. These mutations reduce platelet numbers and cause defects in platelet function.

Mutations that Affect Other Organs

Inherited mutations in GATA2 are associated with MDS and AML starting between ages 10 and 53. Inherited TERT and TERC mutations can lead to dyskeratosis congenita. Inherited SRP72 mutations increase the risk of aplastic anemia and MDS.

To recognize the inherited bone marrow failure syndromes, doctors need to know their signs and symptoms. Doctors must ask patients about their own and their family’s medical history and test patients for inherited mutations that predispose them to bone marrow failure diseases. By identifying these patients early, doctors can provide appropriate care and genetic counseling for patients and family members.
Early Genetic Mutations in MDS

Sidd Jaiswal, MD, PhD
Massachusetts General Hospital

Dr. Jaiswal discussed what happens before a person develops bone marrow failure. Mutations in genes occur throughout life, and most don’t do any harm. But if people live long enough, they can acquire just the right mutation in just the right stem cell to let it reproduce more successfully than normal cells. The cells with this mutation are called “clones,” and this process is called “clonal hematopoiesis” when it occurs in blood stem cells.

At least 10% of everyone over age 70 has a mutation in a gene that causes clonal hematopoiesis. The most commonly mutated genes are DNMT3A, TET2, and ASXL1, but other genes can be mutated as well. Many of these mutations are common in bone marrow cancers. Healthy people with clonal hematopoiesis typically have a mutation in only one of these genes, whereas people with bone marrow cancers usually have mutations in several of them. So the mutations in healthy people might be the first genetic “hit” in the process that can eventually lead to bone marrow cancer.

Indeed, people with clonal hematopoiesis have a higher risk of developing a bone marrow cancer, although the absolute risk is only about 0.5-1% per year. Also, they usually have normal blood counts. Therefore, having a clone with a somatic (not inherited) mutation does not necessarily mean that the person has MDS or another bone marrow failure disease.

Some people with clonal hematopoiesis eventually get bone marrow cancers of myeloid cells, like MDS or acute myeloid leukemia. But some develop a cancer of lymphoid cells. So clonal hematopoiesis is not the same thing as MDS or even pre-MDS. The factors that seem to increase the chance that a person will develop MDS or another type of cancer seem to include the number of cells with the mutation and having more than one mutation.

Several experts have suggested that these findings indicate a new pre-cancerous condition: clonal hematopoiesis of indeterminate potential (CHIP). CHIP is a detectable clonal mutation in the blood of healthy persons who have no known bone marrow disorder. For now, there is no suitable treatment for people with CHIP, so healthy people don’t need to be screened for CHIP.

Splicing Gene Mutations in MDS

Omar Abdel-Wahab, MD
Memorial Sloan Kettering Cancer Center

The genetic information stored in DNA is transcribed into a related molecule, messenger RNA. Messenger RNA contains the genetic coding information needed to make proteins. RNA molecules and proteins splice together certain sequences of RNA to form messenger RNA molecules that can produce proteins that work properly.

Most somatic (not inherited) mutations associated with MDS involve genes that control messenger RNA splicing. About 20% of patients with MDS have mutations in a splicing gene, SRSF2. Patients without this mutation tend to survive longer than those who have the mutation.

In experimental mice, the SRSF2 mutation leads to a disease that is similar to MDS. If mice lose both copies of SRSF2, they can’t make blood cells.

Several other splicing factors are involved in MDS. Mutations in SF3B1, SRSF2, and U2AF1 change the RNA splicing behavior of proteins. But each mutation affects splicing in a different way and is associated with different bone marrow failure diseases. For example, people with a mutation in SRSF2 tend to develop refractory anemia with ring sideroblasts, a type of MDS.

Patients never have mutations in more than one splicing gene because cells can’t survive if they have two of these mutations. Researchers have used this finding to suggest that altering splicing in cells with a splicing mutation, such as MDS cells, might kill the cells. Dr. Abdel-Wahab tested this hypothesis using a drug called E7107 in cells with an SRSF2 mutation. Increasing doses of the drug affected splicing in cells.

A compound that inhibits E7107 in mice with acute myelogenous leukemia and an SRSF2 mutation increased survival to a modest extent. Cells with mutant splicing were more sensitive to this treatment than cells with normal splicing.
Transforming Growth Factor-Beta Signaling in MDS

Amit Verma, MBBS
Albert Einstein College of Medicine

Dr. Verma talked about the role of transforming growth factor-beta (TGF-β), a protein that controls many functions in most cells in the body, in MDS. He also discussed experimental drugs that target TGF-β signaling and could increase blood cell counts in patients with MDS.

The risk of death is higher in MDS than in lung cancer and other solid tumors, even though most cases of MDS are classified as low risk. Most patients with MDS eventually die of low blood-cell counts. These patients need treatments that help their bone marrow make healthy blood cells.

Dr. Verma decided to study TGF-β because it controls blood cell formation in bone marrow. Defective signaling of TGF-β might help explain the inability to form healthy blood cells in MDS.

SMAD genes produce instructions in the TGF-β pathway for making proteins that control the activity of certain genes as well as cell growth and division. Bone marrow samples from patients with MDS have activated SMAD2 and SMAD3.

Levels of SMAD7 are lower in MDS, making cells more sensitive to TGF-β. It is possible to reverse this increased sensitivity by inhibiting TGF-β receptor kinase.

Galunisertib is a TGF-β kinase inhibitor. In a Phase II clinical trial, galunisertib increased blood cell counts in about one quarter of 38 patients with low-risk and intermediate-1-risk MDS. During treatment, the responding patients stopped needing blood cell transfusions. The investigators used a conservative dose of the drug because it can cause side effects. It might be possible to predict which patients will respond to the treatment using a biological marker.

Two other TGF-β family inhibitors, sotatercept and luspatercept, inhibit SMAD2/3 signaling. In two clinical trials, they led to very significant increases in red blood cells in patients with low-risk MDS. An ongoing Phase III trial has started testing the efficacy of luspatercept in patients with MDS with ringed sideroblasts.
MicroRNA Mutations in Bone Marrow Failure

Aly Karsan, MD
University of British Columbia

MicroRNAs (miRs) are small RNA molecules in the genomes of plants and animals that control gene expression by blocking the production of proteins. RNA is a “chemical cousin” of DNA. Dr. Karsan described the role of changes in the level of miRs in controlling cytokines (a type of signaling protein) that suppress both healthy and abnormal blood cell formation in bone marrow. This action has to be reversed for the bone-marrow cells to progress from MDS to AML.

Loss of one of the normal two copies of miR-146a results in inappropriate suppression of immune system signaling through increased expression of tumor necrosis factor receptor-associated factor 6 (TRAF6). TRAF6, a protein, plays a critical role in the proper functioning of the immune system.

When the expression of miR-145 and miR-146a is decreased in mice, about a quarter die of bone marrow failure and a quarter die of leukemia. This effect is due to interleukin-6 (IL-6), which represses both normal blood cell formation and abnormal blood cell formation in mice. Progression to acute myelogenous leukemia (AML) requires removal of the suppressive effect of IL-6.

miR-145 targets toll-interleukin 1 receptor domain containing adaptor protein (TIRAP), which is also part of the immune system. Increasing the expression of miR-145 suppresses the activity of TIRAP, whereas removing miR-145 increases the activity of TIRAP. Interferon gamma, a cytokine, represses normal cells transplanted into mice but also cancerous cells that express TIRAP. It is only when the interferon gamma signal is removed that the cells form too many immature blood cells, a step in the progression of MDS to AML.

Interferon-Gamma and Blood Cell Formation

Martijn A. Nolte, PhD
Sanquin Blood Supply and University of Amsterdam

T cells, which are part of the immune system, seem to play an important role in the formation of healthy blood cells in bone marrow. They produce cytokines (a type of protein). One of these cytokines is interferon-gamma (IFN-G). Dr. Nolte explored the role of IFN-G in blood cell formation. He pointed out, for example, that IFN-G can suppress the formation of healthy blood cells in aplastic anemia.

When a person is infected by a virus, the immune system becomes active. Large numbers of T cells travel to the bone marrow, where they produce large amounts of IFN-G. The production of IFN-G has two different effects. First, IFN-G inhibits the formation of red blood cells in bone marrow. Second, IFN-G increases the clearance of healthy red blood cells from the circulation. Both effects contribute to the development of anemia.

IFN-G inhibits red blood cell formation because it turns on the expression of IRF-1, which in turn increases the expression of PU.1. PU.1 expression in immature bone marrow cells causes these cells to form white blood cells but inhibits the formation of red blood cells. This is because PU.1 inhibits the function of GATA1, which is necessary for the formation of red blood cells. As the amount of IFN-G rises, the bone marrow forms more white blood cells and fewer red blood cells.

Dr. Karsan used these and other findings to describe the early steps in the development of bone marrow failure. First, an MDS cell or clone (copy) becomes abnormal as a result of a genetic mutation. Over time, it acquires more mutations. At first, the cell produces different types of blood cells. But these cells produce cytokines that suppress normal blood cell formation. Eventually, the abnormal cell that started this process spreads from its starting place. It starts suppressing the formation of healthy blood cells in its new place. After some other events, the miR signal that normally suppresses the cytokine is lost and leukemia develops.
Maintaining the right number of red blood cells requires a balance between red blood cell production and clearance. In addition to inhibiting the formation of red blood cells, IFN-G can activate macrophages (large white blood cells). These macrophages increase the removal of red blood cells from the circulation. Anemia is the result.

IFN-G helps the body respond to infections from viruses. When a viral infection happens, white blood cell formation increases temporarily and the bone marrow produces fewer red blood cells. This process helps the immune system fight the virus. But long-term activation of the immune system can lead to anemia.

Darwinian selection means that more advantageous mutations allow some clones to produce more “daughter” clones than clones without the mutation. These mutations become more common as the clone with the original mutation and its offspring reproduce. If this process keeps going, every clone in the bone marrow eventually develops that mutation.

Genetic drift means that just by chance, clones with certain mutations form more daughter clones than others. In genetic drift, wherever clones with the mutant gene spread, new clones have the same mutation. When a population is small, random changes in mutations in clones from one generation to the next can have a major effect on the frequency of mutations.

In addition to Darwinian selection and genetic drift, a third possibility is that clones with mutations can expand if they are in the right environment. This is what happens in paroxysmal nocturnal hemoglobinuria, where an attack on certain cells causes a great deal of damage. The few cells with a mutation are protected from that damage and can still reproduce. Because cells without the mutation are damaged and can’t reproduce, the cells with the mutation eventually take over the bone marrow.

**Non-Inherited Mutations in Bone Marrow Failure Syndromes**

Lucio Luzzatto, MD
Muhimbili University Hospital

Dr. Luzzatto discussed various theories of evolution that could explain how abnormal clones (copies) of immature cells in the bone marrow can reproduce more successfully than healthy clones and eventually take over the bone marrow. This takeover by abnormal clones leads to MDS, paroxysmal nocturnal hemoglobinuria, or aplastic anemia.

Bone marrow stem cells make copies, or clones, of themselves all the time. The cloned stem cells eventually become mature blood cells. The mature cells leave the bone marrow and enter the bloodstream.

A bone marrow failure disease starts when one of these clones becomes abnormal. This abnormal clone makes clones of itself. These “daughter” clones might not be able to make normal blood cells or they might not make as many blood cells as the body needs. Once an abnormal clone develops, its offspring can crowd out normal stem cells and take over the bone marrow.

Everyone develops mutations in their genes throughout life. For every gene in an adult, at least one clone has a mutation in that gene. Some mutations are neutral, meaning that clones with these mutations don’t have any effects. But clones with other mutations grow more quickly than other cells.

Clones with non-inherited mutations can expand in the bone marrow by two types of evolution: Darwinian selection and genetic drift.

Effects of GATA2 Mutations on Blood Cells and the Immune System

Matthew Collin, MD
Newcastle University

A mutation in the GATA2 gene is a rare cause of MDS and acute myelogenous leukemia (AML) that can run in families. Before they get MDS or AML, patients with the GATA2 mutation develop low counts of certain white blood cells that play an important role in the immune system. This type of immunodeficiency is called DCML deficiency, named after the white cells that are missing (dendritic cells, monocytes, and B and natural killer lymphocytes).

Problems due to GATA2 mutation typically start gradually. Children with a GATA2 mutation have normal blood counts and respond normally to infections and vaccines, showing that their immune system is working properly. These people typically start developing troublesome infections and other problems between the ages of 20 and 30 as their immune cell counts begin to drop. Some patients can remain relatively well because they continue to produce antibodies and have normal counts of T lymphocytes. T lymphocytes are a type of immune cell that is less dependent on bone marrow function.
Even when patients remain well, analysis of the bone marrow shows that GATA2 mutation causes failures in the production of immature, B cells, natural killer cells, and dendritic cells. Infections that do occur might speed up the aging of stem cells in bone marrow. In this way, infections can contribute to the progression of disease from an immune cell problem to widespread bone marrow failure and MDS.

Doctors should suspect that a patient with MDS has a GATA2 mutation if the patient is in his or her 20s or 30s and has a relevant family history, signs of infection (such as warts or chest problems), swollen limbs, or autoimmune disease. Lab tests may also show a typical picture of DCML deficiency with low immune cell counts and high levels of Flt3 ligand, a hormone that controls blood cell development. Once MDS has developed, the treatment is the same as for other patients with MDS. But because the GATA2 mutation can be inherited, it is important to screen other family members.
BMT and IST are quite expensive, which is why only a few patients received the standard care in the Philippines. Eltrombopag is a promising new treatment for aplastic anemia, but its cost is probably out of reach for most people in the country.

As of December 2015, 191 (39%) of the 484 patients diagnosed with aplastic anemia between 2010 and 2015 were alive. Another 122 (25%) had died. The status of the remaining 171 patients (35%) is not known.

Dr. Baylon called for more studies of cheaper drugs like cyclosporine and androgen for patients with aplastic anemia in developing countries.

About 70% of patients are very poor and live in rural areas with poor sanitation. Although the cause of aplastic anemia is not usually known, insecticides and fertilizers, indigenous medicines, and lead exposure are thought to play a role in many cases. Patients often have anemia and bleeding at diagnosis. Most cases are severe or very severe.

The only treatment available in Myanmar consists of androgens (usually danazol with or without cyclosporine) and supportive care, including blood transfusions, to treat the symptoms of aplastic anemia. This year, cyclosporine is available because its cost is subsidized. Although danazol with or without cyclosporine has poor outcomes in general, some patients do respond. About 50% of children respond to danazol, and some stop needing regular blood transfusions.

Last year, Dr. Gyi did three bone marrow transplants and she hopes to expand this treatment to more patients with aplastic anemia. She can get government support for bone marrow transplantation for some patients. Dr. Gyi looks forward to the availability of eltrombopag, a new drug that has had promising results in aplastic anemia. Eltrombopag will be more cost effective and suitable for her patients. Also, this drug might have fewer complications than danazol alone or danazol and cyclosporine.

Aplastic Anemia in Myanmar

Aye Aye Gyi, MBBS, MMedSc, DrMedSc, FRCP
North Okkalapa General Hospital, Yangon, Myanmar

The population of Myanmar is over 55 million, but the country has only three adult hematology centers and two pediatric oncology centers. Myanmar has less than a dozen qualified hematologists, including current trainees. General physicians help treat aplastic anemia.

Between 1996 and 2010, the number of cases of aplastic anemia increased every year at Yangon General Hospital, which is the country’s main referral hospital. The total number of aplastic anemia diagnoses during this period was 1,042, and the median age at diagnosis was 32. At North Okkalapa General Hospital, the number of cases of aplastic anemia also increased between 2012 and 2015. Mortality rates are high particularly for very severe aplastic anemia. The rate of new cases of aplastic anemia per year is probably higher than 10 cases per million people in some areas of Yangon Division with at least 100,000 people.
March 18, 2016

TRANSPLANT TREATMENTS FOR BONE MARROW FAILURE

Session Co-Chairs:
Carlo Dufour, MD
Giannina Gaslini Children’s Hospital
Mary Eapen, MD, MS
Medical College of Wisconsin

Stem Cell Transplantation for Older Patients with Severe Aplastic Anemia

Judith Marsh, M.D.
King’s College Hospital

Dr. Marsh discussed the outcomes of hematopoietic stem cell transplantations (HSCT) in older patients with aplastic anemia as well as ways to improve these outcomes. Survival after HSCT from unrelated donors whose stem cells match those of the patients has increased significantly in recent years. People in their late 60s may now be able to have HSCT, and 57% of those who have HSCT are older than 40.

The treatment strategies for severe aplastic anemia are well developed for younger patients but less for those older than 60. Dr. Marsh recommends first finding out if the patient is eligible for treatment based on his or her health and ability to function. If the patient is eligible for treatment, the best option depends on how severe the aplastic anemia is and whether the patient has an appropriate HSCT donor. Other treatment options include drugs that suppress the immune system. If the patient isn’t eligible for treatment, care focuses on keeping the patient as comfortable as possible with best supportive care.

The limited evidence shows that older patients don’t do as well after HSCT than younger patients. But older patients might do better with different conditioning treatment to prevent their immune system from attacking the transplanted cells and to prevent the new immune cells from the donor from attacking the patient’s body. For example, older patients seem to be more likely to survive after HSCT if their conditioning treatment includes a lower dose of cyclophosphamide than usual.

Unrelated Donor Transplantation for Children with Severe Aplastic Anemia

Sujith Samarasinghe, MBBS, PhD
Great Ormond Street Hospital

Dr. Samarasinghe discussed hematopoietic stem cell transplantation (HSCT) and immunosuppressive treatment (IST) in children and teens with aplastic anemia. The first choice treatment for severe aplastic anemia is hematopoietic stem cell transplant (HSCT) from a sibling whose HLA markers match the patient’s. If the patient doesn’t have a matched sibling donor, the second choice is immunosuppressive treatment (IST) or, for a child, HSCT from a matched unrelated donor.

Rates of survival for at least 10 years after matched sibling HSCT in children with severe aplastic anemia used to be much higher than after IST. Today, many children and teens survive as long with IST as with HSCT. But aplastic anemia is less likely to progress to MDS or leukemia if the first treatment is HSCT. Also, response rates to IST are a bit lower than with HSCT, and children are more likely to need a different treatment if their disease progresses, which could affect their quality of life.
Eltrombopag, a new drug, could be a game changer for aplastic anemia, and it could change decisions about unrelated donor HSCT in the future. Patients treated with eltrombopag have much better response rates than those treated with IST. But longer-term follow-up data are needed to track whether aplastic anemia progresses to MDS or leukemia over the long run.

Survival rates of HSCT with matched unrelated donors are now almost as good as with matched siblings, at least in children. Children who haven’t responded to IST tend to do very well after HSCT from an unrelated donor. Their aplastic anemia rarely progresses to MDS or leukemia, and they rarely get chronic graft-versus-host disease, a serious complication of HSCT.

In the United Kingdom, matched unrelated donor HSCT is an option for children and teens with aplastic anemia who don’t have a matched sibling donor. Doctors try to decide whether to move forward with an unrelated donor transplant within 2 months of diagnosis after discussing the pros and cons with the patient and family. But finding a matched unrelated donor can be challenging for patients with certain ethnic backgrounds, such as blacks with South American or Central American ancestry.

Dr. Samarasinghe and colleagues now used matched sibling HSCT to treat newly diagnosed children and teens with severe aplastic anemia. The second choice is matched unrelated donor HSCT or IST. If the choice is IST and it doesn’t work, the next step is HSCT if a donor is available.

**Transplantation for Fanconi Anemia**

*Dr. Farid Boulad, MD*
Memorial Sloan Kettering Cancer Center

This presentation focused on the use of hematopoietic stem cell transplantation (HSCT) to treat Fanconi anemia, the most common inherited bone marrow failure disease. The average age at diagnosis with Fanconi anemia is 7 years. By age 10, three quarters of these patients develop aplastic anemia, and by age 40, half develop MDS or acute myelogenous leukemia.

HSCT is the treatment of choice in patients with Fanconi anemia who develop low counts of one type of cell or all three types of cells (severe aplastic anemia), high-risk MDS, or aplastic anemia.

In the 1980s, few patients with Fanconi anemia survived after HSCT because of complications. In the 1990s, HSCT started working well for patients with Fanconi anemia who had a sibling with blood markers that matched those of the patient. By that time, doctors had started transplanting bone marrow from unrelated donors. But only one in three patients did well because of several complications that included organ toxicity (a life-threatening side effect of chemotherapy), graft-versus-host disease (GVHD), graft rejection, and infections.

By 2000, transplants for patients with Fanconi anemia started to work much better even when the bone marrow came from unrelated donors. The reasons included the use of fludarabine (Fludara), a new medication, to prevent the immune system from attacking the transplanted cells. Another important advance was the manipulation of the stem cells by removal of the donor’s immune cells (T-cell depletion) to prevent the GVHD complications.

The next goals for transplant doctors are to try to do transplants for patients with Fanconi anemia using mismatched donors in the patient’s family and to improve the results by eliminating the last two complications, organ toxicity and infections.

**Long-Term Outcomes after Transplantation for Fanconi Anemia**

*John E. Wagner, MD*
University of Minnesota

Dr. Wagner described what happens over the long term to patients with Fanconi anemia after allogeneic hematopoietic stem cell transplantation (HSCT).

In 1995, HSCT for Fanconi anemia was associated with poor survival, especially for patients whose donor wasn’t a family member. Today, regardless of the type of stem cell donor, the survival rate is 90% or better if the patient has the transplant before developing major infections, needing regular transfusions, or developing advanced-stage MDS or leukemia.

The complications of HSCT from related donors are much less serious if the sibling’s HLA markers match all of the patient’s HLA markers. For this reason, some mothers of children with Fanconi anemia choose to become pregnant using in vitro fertilization and preimplantation genetic diagnosis to find out which embryos are free of Fanconi anemia and have matching HLA markers to the patient. In other words, these women give birth to a “savior sibling.”
Dr. Wagner and his colleagues continue to offer this approach because it leads to a better survival rate and decreases the risks of HSCT.

Some serious side effects of HSCT can happen weeks or months after the procedure. These complications can include low hormone levels, cataracts, bone and joint problems, and infertility. Strategies to reduce these risks include using lower amounts of steroids and shielding the ovary during the procedure.

In the 1990s, up to 45% of patients developed cancer after HSCT, especially if they had chronic GVHD. However, cancer risk is also high in patients with Fanconi anemia who haven’t had HSCT. This finding suggests that DNA repair defect that is characteristic of Fanconi anemia plays a role in cancer risk in these patients. As the doses of chemotherapy and radiation used with HSCT have dropped and the ability to prevent GVHD has improved, the cancer risk seems to be lower today. At the University of Minnesota, similar proportions of patients with Fanconi anemia develop cancer by age 45 regardless of whether they have had HSCT.

Today, patients with Fanconi anemia can expect to survive for a long time after HSCT. The greatest threat to their long-term survival is the high risk of cancer. So centralized repositories of data and specimens are urgently needed to help researchers better understand why cancer develops in patients with Fanconi anemia and how to best treat cancer once it occurs.
with IST alone in the past, those in this study were more likely to respond, had greater increases in white blood cell and platelet counts, and their blood cell counts recovered very quickly. Most patients stopped needing regular red blood cell transfusions within about a month. For all 92 patients, the survival rate after a median of 18 months is close to 100%.

Two patients got severe rashes, but there were no other major side effects. One patient died during the study of encephalopathy (brain malfunction) related to a concurrent cancer of the thymus gland that existed before treatment for aplastic anemia. Two others died after bone marrow transplantation due to acute myelogenous leukemia or relapsed aplastic anemia. Five patients developed MDS, and four of these patients had immediate bone marrow transplantation.

Phase II Clinical Trials of Eltrombopag for Aplastic Anemia

Dr. Winkler described several clinical trials of eltrombopag (Promacta). He first talked about a Phase II clinical trial in 43 patients with severe aplastic anemia who had not responded to immunosuppressive treatment. The investigators assessed the effect of the treatment on blood cell counts after 12 weeks of treatment. Patients could continue the eltrombopag treatment until they had a clear response or their blood cell counts didn’t improve further.

Seventeen patients (40%) responded to eltrombopag. At 12 weeks, responding patients had higher counts of at least one type of blood cell, but only one patient had higher counts of red blood cells, white blood cells, and platelets. Blood cell counts increased after eltrombopag treatment ended in two patients who didn’t respond within the first 4 months. Several nonresponders had a meaningful biological response but didn’t meet the study’s definition of response by 12 weeks.

These findings led to an ongoing follow-up study to assess the effects of longer-term eltrombopag on blood counts in patients with aplastic anemia that hasn’t responded to other treatments. Patients were treated with 150 mg eltrombopag a day for 6 months. Responding patients could then continue eltrombopag treatment until they had a robust response or no further improvement in blood cell counts over at least 6 months.

Of the first 36 patients in the study, 17 (47%) responded to eltrombopag by 6 months. Six responders had marked increases in all blood cell types, and six others had increases in at least two types of blood cells. Five nonresponders had to stop taking eltrombopag because of abnormal chromosomes. Three patients chose a different treatment before they could be evaluated at 6 months. Five patients who didn’t respond at 3 months did respond at 6 months. One patient developed MDS and acute myelogenous leukemia within 3 months and died. Among 16 responders who continued taking eltrombopag after 6 months, 9 had a robust response after a median of 15 months.

Dr. Winkler also presented preliminary results of another clinical trial of eltrombopag in patients with moderate aplastic anemia. Doses are increased every 2 weeks until the maximum dose of 300 mg a day. Of the 21 patients whose results could be evaluated so far, 11 (52%) have responded. Two of five patients with robust responses have had a relapse, but their blood cell counts recovered after eltrombopag was restarted. Three patients developed severe aplastic anemia and responded to standard immunosuppressive therapy with horse antithymocyte globulin and cyclosporine plus eltrombopag.

Genetic Causes of Outcomes in Bone Marrow Failure Diseases

Dr. Maciejewski talked about the use of data on genetic mutations in patients with MDS and aplastic anemia to predict outcomes. People with MDS have many different types of abnormalities in their genes and chromosomes. For example, they might have both inherited and non-inherited mutations in genes. Some of these mutations involve changes in DNA; others don’t. Because these diseases are so complex, even the genetic mutations found most often in people with MDS and other bone marrow failure diseases aren’t usually all that common.

Some mutations are known to affect survival. For example, patients with 5q-syndrome, a type of MDS, who have a mutation in TP53 survive longer than patients without this mutation. But this type of analysis is hard to do with uncommon genetic mutations.

In people with MDS, bone marrow cells with genetic
mutations can make abnormal copies (clones) of themselves that can reproduce more easily than healthy cells. These abnormal clones take over much of the bone marrow, resulting in shortages of healthy blood cells.

Patients with MDS acquire different mutations at different stages in the evolution of clones. For example, the original (founding) clone might have certain inherited or non-inherited mutations. The “daughter” subclones formed by the founding clone can develop new mutations.

Based on the mutations in subclones, researchers can sometimes predict which patients with MDS are likely to develop leukemia and which ones aren’t. They can also use findings on which mutations happened first to predict survival. This information is useful for choosing the right treatments and managing the disease.

The secondary MDS that evolves from aplastic anemia has different genetic features from primary MDS, meaning that the patient hasn’t had aplastic anemia or another type of cancer before. For example, patients whose aplastic anemia evolves to MDS are less likely to have mutations in TET2, SF3B1, and ASXL1 than those with primary MDS.

In a study in 13 patients with aplastic anemia treated with eltrombopag (Promacta) at the Cleveland Clinic, 6 patients responded to the treatment. However, a few patients had expansions of abnormal clones with mutations that might be associated with progression to leukemia during eltrombopag treatment.

An Update on European Studies of Eltrombopag for Aplastic Anemia

**Antonio Risitano**, MD, PhD
University of Naples

Dr. Risitano discussed two ongoing European clinical trials on eltrombopag (Promacta) in patients with aplastic anemia.

The first trial is called Eltrombopag for Moderate Aplastic Anemia. This study randomly assigns patients to treatment with a combination of cyclosporine and eltrombopag or a combination of cyclosporine and placebo. Within the two treatment groups, patients are divided into those who need regular blood transfusions and those who don’t, as well as those who are 60 or younger and those who are older than 60.

The starting dose of eltrombopag is 150 mg a day, and the daily dose can go up to 225 mg after 3 months if the patient hasn’t responded in a satisfactory way yet. The study is recruiting patients in France, Germany, United Kingdom, Italy, the Netherlands, and Switzerland.

To be eligible for the study, patients can’t have been treated for aplastic anemia before. The investigators will look for responses after 6 months of treatment. At that time, they might continue the treatment for up to 6 more months.

The second trial is a Prospective Randomized Multicentre Study Comparing Horse Antithymocyte globulin (hATG) + Cyclosporine A (CsA) + Eltrombopag.

This trial, known as RACE, aims to improve the quality and speed of response in patients with severe aplastic anemia. The ultimate goal is to provide the evidence needed to establish a new standard of care for this condition. The study is randomly assigning patients with severe or very severe aplastic anemia to treatment with hATG, cyclosporine, and eltrombopag or hATG and cyclosporine only. This study is not using any placebo.

All patients in this Phase III randomized clinical trial get the same dose of eltrombopag, 150 mg a day. If patients are in remission after 3 months, they will stop taking the eltrombopag. Those who aren’t in remission will continue taking the eltrombopag for 3 more months. Patient responses will be assessed one last time at 6 months. The study has sites in France, Germany, Italy, the Netherlands, Spain, Switzerland, and the United Kingdom.

Eltrombopag for Aplastic Anemia in Developing Countries: The Brazilian Experience

**Phillip Scheinberg**, MD
Hospital São José and Beneficência Portuguesa de São Paulo

Treatment of aplastic anemia with antithymocyte globulin (ATG) weakens the immune system and stops it from attacking the bone marrow. ATG can be made from horses or rabbits, but rabbit ATG doesn’t seem to work as well as horse ATG.

Horse ATG is available in the United States and some European countries. But rabbit ATG is the only form of ATG available in many countries that have high rates of aplastic anemia, including Brazil. And some developing countries don’t even have access to rabbit ATG. Other disadvantages of ATG are that it has to be given by
intravenous infusion (through a catheter), it can have side effects, and older patients are less likely than younger patients to tolerate and respond to it.

Cyclophosphamide is another treatment used for aplastic anemia in developing countries. But less than half of patients respond to it and many develop a severe infection or die within 6 months of starting this treatment.

Dr. Scheinberg is part of the Brazilian Marrow Failure Network, which is studying the use of eltrombopag (Promacta) and cyclosporine for severe aplastic anemia. Eltrombopag is an appealing option because it might allow developing countries to avoid the downsides of rabbit ATG or cyclophosphamide.

The leaders of this study hope that the combination of eltrombopag and cyclosporine will improve outcomes in patients with aplastic anemia in regions of the world that don’t have access to intravenous treatments. If this combination is effective in aplastic anemia, it could overcome the shortcomings of ATG (e.g., side effects, low response rate of rabbit ATG, and lack of access to horse ATG in most parts of the world) by providing an alternative all-oral outpatient regimen with fewer side effects.

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**NON-TRANSPLANT TREATMENTS FOR MDS**

**Session Co-Chairs:**
Luca Malcovati, MD
University of Pavia
David Steensma, MD
Dana-Farber Cancer Institute

**Where Do TPO Agonists Fit in MDS**

Mikkael Sekeres, MD, MS
Cleveland Clinic

Dr. Sekeres described the National MDS Natural History Study. This study will follow 2,000 patients with MDS and 500 with idiopathic cytopenia of undetermined significance for the rest of their lives. The study will collect medical information and biological specimens.

One study goal is to learn about biological and genetic factors involved in the development and progression of MDS. The study will also shed light on changes in bone marrow stem cells in MDS; find biological markers of disease onset, progression, or response to therapy; identify potential targets for new treatments; and improve the management of MDS.

Dr. Sekeres also discussed the role of thrombopoietin agonists (TPO) in MDS. TPO is a hormone that controls platelet production in the bone marrow. By stimulating TPO, TPO agonists increase the number of platelets and decrease bleeding risk. Dr. Sekeres recommends TPO agonists or platelet transfusions for patients with lower-risk MDS who have a low platelet count.

In a randomized controlled trial, romiplostim (Nplate), a TPO agonist, increased platelet counts in patients with lower-risk MDS. Unfortunately, rates of progression of MDS to acute myelogenous leukemia (AML) at the end of the study were 2.5 times higher than in the placebo group. However, 5 years later, rates of progression to AML and rates of AML-free survival were the same in both the romiplostim and placebo groups. Patients who have low TPO levels and need fewer units of transfused patients before treatment are most likely to respond to romiplostim.
Only a few studies have evaluated the use of eltrombopag (Promacta), another TPO agonist, in MDS. In two studies in patients with lower-risk or intermediate-risk MDS who had severe platelet shortages, eltrombopag significantly improved platelet counts. Patients tolerated the treatment well.

Dr. Sekeres concluded that TPO agonists are useful for treating platelet shortages in patients with lower-risk MDS. But they don’t prolong survival and shouldn’t be used in anyone who has excess blasts (immature bone marrow cells). The value of combinations of TPO agonists with other treatments still needs to be determined.

**Genetic Testing to Predict Responses to MDS Treatment**

**Rafael Bejar**, MD, PhD
University of California, San Diego

Predicting how a patient will respond to a drug before treatment can be useful for preventing serious side effects in patients who aren’t likely to respond to that therapy. Predicting early on during treatment how patients might eventually respond to a treatment, or whether a patient who has responded to a treatment will have a relapse, is also useful.

Lenalidomide (Revlimid) is a biologic agent that is used successfully to treat lower risk MDS with 5q deletion. Patients with this type of MDS have a deletion (loss) of the long (q) arm of chromosome 5 either by itself or along with another chromosomal abnormality. Responses to lenalidomide in patients with lower-risk MDS who don’t have 5q deletion MDS are much more modest. So 5q deletion is a useful biological marker to predict responses to lenalidomide.

Patients with 5q-deletion MDS often have mutations in the TP53 gene. TP53 mutations are markers of shorter survival. These mutations might be markers of relapse in patients with 5q-deletion MDS who are treated with lenalidomide.

Some evidence suggests that TET2 mutations might predict a greater chance of responding to azacitidine (Vidaza) in treated patients with MDS, especially if these patients don’t have an ASXL1 mutation. However, patients with TET2 mutations don’t necessarily live longer than patients without these mutations. In some studies, mutations in TP53 and PTPN11 seemed to predict shorter survival after azacitidine or decitabine (Dacogen) treatment. In other studies, mutations in ASXL1 and EZH2 seemed to predict longer survival after azacitidine treatment.

Dr. Bejar concluded that somatic (non-inherited) mutations aren’t strong markers of response to azacitidine or decitabine. So he doesn’t recommend that doctors use genetic test results to justify not treating patients who have MDS with these drugs. Tracking mutations might not be useful for predicting responses to treatment because mutations can persist even in patients who respond. But tracking mutations might help identify early relapses in patients who do respond.

**MDS Treatments that Target Tumor Growth Factor-Beta Signaling**

**Uwe Platzbecker**, MD
University Hospital Carl Gustav Carus Dresden

Dr. Platzbecker discussed a novel approach to treating anemia associated with MDS. In MDS, the bone marrow produces many immature stem cells that don’t mature into healthy red blood cells. This is known as “ineffective erythropoiesis,” and it results in anemia (reduced number of healthy red blood cells). Anemia is a hallmark of MDS, and most patients with MDS have anemia.

Various proteins control red blood cell production, or erythropoiesis. Erythropoietin stimulates production of immature red blood cells. But stimulating this pathway with erythropoiesis-stimulating agents doesn’t always work in MDS. Other proteins, especially members of the TGF-β family, slow the bone marrow’s production of red blood cells. These proteins might contribute to the anemia in people with MDS. Targeting these proteins could release the “blockage” in the bone marrow and help promote the production of mature red blood cells.

Luspatercept is an experimental drug that increases red blood cell and hemoglobin levels by blocking the activity of proteins in the TGF-β superfamily. A phase 2 clinical trial has shown that luspatercept increases hemoglobin levels and can reduce or eliminate the need for red blood cell transfusions in patients with lower-risk MDS.
In 47% of patients with a low transfusion burden (those needing less than 4 units over 8 weeks) treated with luspatercept, hemoglobin levels dropped by at least 1.5 g/dL. Many patients sustained their hemoglobin increase for as long as 9 months (the study is still ongoing). In patients with a high transfusion burden (those needing more than 4 units over 8 weeks), 50% of those treated with luspatercept needed at least 4 fewer units and 25% stopped needing transfusions for at least 8 weeks. Longer treatment of patients who responded maintained these benefits for as long as 9 months. Finally, patients with certain biological markers measured at baseline had higher response rates.

Dr. Platzbecker concluded that luspatercept has shown encouraging clinical effects in patients with MDS. The drug also has a good safety profile. Based on these results, an international Phase 3 clinical trial—the MEDALIST Trial—of luspatercept has started in patients with lower-risk MDS.

Advances in Treatment of Non-5q Deletion Lower-Risk MDS

Azra Raza, MD
Columbia University Medical Center

Dr. Raza described predicting survival in patients with MDS as very challenging, making it difficult to offer each patient just the right treatment.

Doctors can use the Revised International Prognostic Scoring System (IPSS-R) to predict survival and likelihood of developing acute myelogenous leukemia (AML). According to the IPSS-R, patients with very low-risk MDS survive an average of 8.8 years after diagnosis, whereas those with very high-risk MDS survive an average of just 0.8 years. The IPSS-R predicts that 13% to 31% of patients with MDS will die of AML, depending on their risk group. Of those who die soon after diagnosis, 70% have bone marrow failure and profound shortages of certain types of blood cells.

Lenalidomide (Revlimid) is a biologic agent that slows down the growth of blood vessels that feed abnormal cells. This drug can eliminate the need for red blood cell transfusions in patients with 5q deletion MDS. Clinical trials have shown that about 27% of patients with non-5q deletion, lower-risk MDS stop needing red blood cell transfusions when they are treated with lenalidomide.

The combination of lenalidomide with erythropoietin, a hormone that helps the bone marrow form red blood cells, seems to be more effective in non-5q deletion, lower-risk MDS than lenalidomide alone. In a Phase III clinical trial in 131 patients needing regular red blood cell transfusions, 39% of those treated with the combination stopped needing blood transfusions compared to 23% of those treated with lenalidomide alone.

In patients with MDS, a chemical process known as ‘methylation’ blocks DNA’s ability to control cell growth. The hypomethylating agents (HMAs) azacitidine (Vidaza) and decitabine (Dacogen) inhibit methylation so that DNA sequences can act normally. In a study of 88 patients with non-5q deletion, lower-risk MDS, 24% stopped needing red blood cell transfusions after treatment with azacitidine or decitabine. Low doses of HMAs seem to be safe and effective in these patients.

In spite of these advances, most patients with non-5q deletion, lower-risk MDS need regular red blood cell transfusions either continuously or from time to time. This is an area of unmet need that requires more research.
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