Chapter 3

Clinical Care of Fanconi Anemia Hematologic Issues

Introduction

Most patients with Fanconi anemia (FA) commonly develop hematologic complications that are primarily related to bone marrow failure (BMF). It is thought that the cause of BMF in patients with FA is a faulty DNA repair pathway that damages hematopoietic stem cells (HSCs) (see Chapter 1). This chapter provides an overview of hematologic care for patients with FA, including guidelines for clinical monitoring of patients and the decision process for determining the need for hematopoietic cell transplant (HCT), the only proven curative treatment for BMF. The chapter also outlines HCT care guidelines and provides a discussion of recent advancements in HCT protocols that have led to significant improvements in the survival rates of patients with FA. Alternative therapeutic options beyond HCT, such as gene therapy, also are discussed.

Bone Marrow Failure

Bone marrow failure (BMF) in patients with FA can range from mild, asymptomatic cytopenias to severe aplastic anemia (AA), myelodysplastic syndrome (MDS), or acute myelogenous leukemia (AML). The absence of BMF, however, does not exclude the diagnosis of FA. More than 90% of patients with FA will have macrocytosis starting in infancy or childhood. However, macrocytosis may be masked by concomitant iron deficiency or an inherited blood disorder such as alpha- or beta-thalassemia trait, which can delay diagnosis of FA [1-3].

Definition of Bone Marrow Failure

Bone marrow failure is diagnosed by blood counts that are below standard age-appropriate ranges. While many patients progress to frank aplastic anemia, others may maintain mildly abnormal blood counts for years and even decades. Bone marrow failure is classified into three broad categories depending upon the degree of cytopenia(s) observed (see Table 1). The classification defines points at which different clinical management options should be considered. Importantly, to meet these criteria for BMF, the cytopenia(s) must be persistent and not transient or secondary to another treatable cause, such as infection, medication, peripheral blood cell destruction or loss, or nutritional deficiencies.

Table 1. Severity of bone marrow failure.

	Mild	**Moderate (or hypoplastic or aplastic anemia)**	**Severe (or severe aplastic anemia)**
Absolute neutrophil count (ANC)	<1,500/mm^3	<1,000/mm^3	<500/mm^3
Platelet count	150,000-50,000/mm^3	<50,000/mm^3	<30,000/mm^3
Hemoglobin (Hb) level	≥8 g/dL*	<8 g/dL	<8 g/dL

**Less than normal for age but > 8 g/dL.*

Bone Marrow Failure Age of Onset

The age of onset of BMF in patients with FA is highly variable. Three out of every four patients develop evidence of at least mild BMF within the first decade of life [3-6]. In an analysis of 754 patients in the International Fanconi Anemia Registry (IFAR), the average age of onset was 7.6 years. However, that study analyzed patients who mainly had defects in the *FANCA*, *FANCC*, and *FANCG* genes; therefore, the results may not be representative

of patients with rarer gene defects [5]. In adults, FA is less commonly diagnosed due to primary BMF; instead, diagnosis of FA more commonly occurs as a consequence of presentation with cancer or with severe toxicity after chemotherapy treatment for a malignancy [4-7]. Severe, usually transient, BMF also may develop in non-transplanted female patients with FA during pregnancy.

The cytopenia that most commonly leads to the diagnosis of FA is thrombocytopenia with red blood cell macrocytosis and elevated levels of fetal hemoglobin. When a patient is diagnosed with FA, or when blood counts fall further, a thorough hematologic workup is necessary to rule out additional causes of cytopenias other than primary BMF. Marrow cellularity must be interpreted in the context of changes in peripheral blood counts as it may be variable and subject to sampling variation. Therapeutic intervention should not be based on bone marrow cellularity alone in the absence of clinically significant peripheral cytopenias or clear evidence (usually clonal cytogenetic changes) of a myelodysplastic or malignant process.

Clinical Monitoring of Bone Marrow Failure

Clinical surveillance and therapeutic management are guided by the following:

- Severity of the cytopenia(s)
- Stability or trend of the peripheral blood counts
- Presence or development of morphologic (dysplastic) and cytogenetic bone marrow abnormalities
- Presence of potentially high-risk genotypes (see Chapter 2)
- Other organ system problems
- Patient's quality of life
- Preferences of the patient and their family

At diagnosis, a trephine bone marrow biopsy should be performed in FA patients to evaluate bone marrow cellularity and architecture, and as an aspirate to assess morphology for dysplastic changes and cytogenetics for abnormalities common to FA and MDS (see Chapter 2). Subsequently, annual evaluation of the bone marrow, beginning at age two, allows for serial comparisons of a patient's marrow, and prompt detection of bone marrow progression that may suggest consideration of transplantation.

Recommendations for Clinical Monitoring of Bone Marrow Failure

Peripheral blood counts stable at no more than mild BMF range (Table 1) and no clonal cytogenetic abnormalities present

- For patients with normal blood counts or mild BMF and no cytogenetic clonal marrow abnormalities, peripheral blood counts and differential white blood cell counts should be reviewed every 3-4 months.
- Consider a bone marrow biopsy and aspirate with cytogenetics annually.

Peripheral blood counts stable in the normal to mild BMF range (Table 1) and clonal cytogenetic abnormalities present

- Blood counts and physical findings should be reviewed every three months for patients with a cytogenetic clonal marrow abnormality (in the absence of morphologic MDS) together with normal or mildly low, but stable, blood counts.
- Bone marrow examination should be performed every 3-6 months to evaluate if the patient's status is stable or changing.
- Review appropriate plans for hematopoietic cell transplantation.

Peripheral blood counts falling or rising

- Patients with progressively changing blood counts without a clinically apparent underlying cause (e.g., transient response to an acute infection or suppression secondary to medication) require immediate evaluation by bone marrow biopsy and aspirate with cytogenetics.
- Rising peripheral blood counts may be due to either the development of MDS or AML (requiring discussion of urgent transplantation) or, rarely, reversion of a germ-line mutation in a stem cell called somatic stem cell mosaicism, which repopulates the marrow with normal cells (see Chapter 2). Such patients require ongoing close monitoring, including blood counts every 1-2 months and a bone marrow examination every 3-6 months.
- Discuss and prepare appropriate plans for HCT intervention as adverse clonal progression or worsening BMF may evolve rapidly.

Clonal Abnormalities

The bone marrow of patients with FA can exhibit dysplasia, such as nuclear/cytoplasmic desynchrony, hypolobulated megakaryocytes, and binucleated erythroid cells. These features are difficult to distinguish from truer forms of MDS and the precise diagnosis determines the need for and type of treatment. The presence of dysplasia is not necessarily a harbinger of MDS and AML; therefore, it is important for FA patients to have a baseline bone marrow examination at diagnosis and regular bone marrow cytogenetic analyses for follow up. Bone marrow examination (which includes aspiration, biopsy, and cytogenetic analysis as described in Chapter 2) should be performed by an experienced hematopathologist at the time of diagnosis and in subsequent serial marrow examination annually. The purpose of the serial marrow examination and cytogenetic analysis is to identify clonal evolution to MDS or AML in the context of peripheral blood count changes or physical exam findings. The results of cytogenetics analyses of patients with FA have revealed varying types and frequencies of clones. An early analysis from the International Fanconi Anemia Registry (IFAR) found that the risk of developing MDS or AML within three years after the observation of a clone was approximately 1 in 3 (35%), whereas the risk for patients without a clone was 1 in 30 (3%) [8]. In another cohort, clones were noted to disappear permanently or reappear in serial marrow evaluations [9].

Gain of 1q (1qG) and/or 3q (3qG), and loss of 7 (7L) comprise the majority of the clonal abnormalities seen in cells from patients with FA [10-13]. The prognostic role of 3qG for predicting progression to MDS or AML was first reported in 18 patients where the three-year risk of MDS/AML was 9 in 10 (90%), compared with 1 in 10 (10%) for patients without aberrations in chromosome 3 [13]. In other studies, the prognostic role of a 3qG has been more difficult to establish. For example, in a study of 119 patients with FA, 32% had clonal aberrations and 20 out of 119 had 3qG [14], although the prognostic power of 3qG could not be evaluated because the chromosome aberration occurred simultaneously with the diagnosis of MDS [14]. Vundinti et al. showed that 10 patients with FA without 3q aberrations progressed to MDS or AML and five of these patients developed other clones [15]. Mehta et al. showed that four of 64 FA patients without MDS and six of 13 with MDS/AML had 3qG, but there was no significant association identified between 3qG and risk of MDS/AML [10]. The results from these studies indicate that 3qG is a common chromosomal abnormality in FA and may be associated with MDS and/or AML, although its prognostic significance is not entirely clear, particularly when it occurs in isolation and different cytogenetic methodologies are used for analysis.

Similar to the non-FA population, the appearance of monosomy 7 and most 7q deletions (7L) is generally associated with a poor prognosis and high risk of developing MDS or AML, whereas trisomy of 1q has not been convincingly shown to associate with prognosis. However, longitudinal prospective studies of larger numbers of patients are required to clarify the prognostic role of specific types of clones and combinations of aberrations.

Physicians must be cautious and assess the latest literature when treating a patient who has a clone but lacks other abnormalities of blood counts or myelodysplastic changes in the marrow. Despite the presence of a clone, some patients may have stable hematopoiesis and possibly a relatively favorable long-term prognosis. Regular marrow examinations before blood count changes offer the best opportunity to diagnose marrow progression and time to discuss treatment options. The interpretation of specific chromosomal abnormalities indicative of clonal progression to MDS and AML is discussed in detail in Chapter 2. The decision on how to proceed should be made by the patient and their family in discussion with an FA physician specialist.

Treatment Guidelines for Patients with Myelodysplastic Syndrome or Acute Myeloid Leukemia

There is no standard therapy for FA patients with MDS or AML. Treatment options include HCT with or without prior induction chemotherapy, and phase I/II trials for MDS or AML. Chemotherapy treatment should be undertaken by centers experienced with FA. Because chemotherapy may cause severe, prolonged, or even irreversible myelosuppression in patients with FA, back-up plans for potential stem cell rescue should be considered. If patients have MDS or AML at the time of their diagnosis with FA, then low-intensity chemotherapy may be used to prepare the patients for transplantation. Published reports

of chemotherapy regimens for AML in patients with FA are sparse and limited by the lack of longitudinal follow-up. It remains unclear whether chemotherapy prior to transplant improves or worsens outcomes [16].

Treatment Options for Bone Marrow Failure

Transfusions

The onset of anemia in patients with FA is insidious. Hemoglobin levels should be monitored closely, at least every 3-4 months from diagnosis, so that treatment may be instituted before transfusion with packed red blood cells is required. Treatment of anemia should be considered when the patient's hemoglobin level consistently falls below 8 g/dL or the patient has other heart or lung disease that requires a higher hemoglobin. Transfusion use should be minimized particularly if the treatment goal is HCT.

All FA patients should receive red blood cells that have been filtered to deplete leukocytes to reduce the risk of cytomegalovirus (CMV) infection. Some centers only use red blood cells that are CMV-negative, whereas most accept leukocyte depletion as an equally effective alternative to CMV-negative products. Irradiated blood products should be used to avoid transfusion-associated graft-versus-host disease (GvHD), particularly if transplant is being considered. Extended antigen matching of transfused red blood cells may be important for patients in certain racial groups for whom minor antigen mismatch is more commonly encountered. Patients should not receive blood transfusions from related family members due to the risk of developing alloimmunization that would increase the risk of graft rejection after HCT. Blood from unrelated designated donors offers no increase in transfusion safety and may delay needed transfusion.

Hematopoietic Cell Transplant

The only curative option for bone marrow failure (BMF) in patients with FA is a hematopoietic cell transplant (HCT). Survival outcomes following HCT have improved significantly for patients with FA primarily due to earlier referral for HCT prior to the onset of MDS and/or AML, refinements in treatment plans and HLA-matching between the patient and donor, and better supportive care before, during, and after HCT.

Recent Advancements in Hematopoietic Cell Transplant

From the institutional and registry studies performed to date, six recent developments have occurred:

- Survival rates after HCT continue to improve, particularly for patients undergoing alternate donor transplant.
- Transplant from related and unrelated donors has similar outcomes.

- Transplant without radiation can be successful for patients with FA.
- Radiation during transplant is clearly associated with increased risk of later cancer in larger series of persons without FA. More studies are needed to determine if radiation or a radiation-free conditioning protocol increases the risk of cancer in patients with FA.
- For patients without a 10/10 or 9/10 matched related donor, 10/10 or 9/10 matched adult unrelated donor and 10-8/10 (or perhaps less) matched umbilical cord blood (UCB) are associated with good outcomes.
- Haploidentical transplant can be successful in FA patients with no other donor option.
- In transplanted FA patients, development of chronic or acute GvHD increases the risk of later cancer.

Sibling Donor Hematopoietic Cell Transplant

In the past, limited field total body irradiation (TBI) was given to FA patients with a human leukocyte antigen (HLA)-identical sibling donor; however, today with the use of fludarabine (FLU) conditioning containing regimens, TBI is used much less often for FA patients with BMF who have HLA-matched sibling donors [17]. Bonfim et al. reported that 85 patients with FA (median age 9 years, range 3-34 years) and a matched sibling donor have been transplanted using a radiation-free regimen [18]. The treatment consisted of cyclophosphamide (CY) (15 mg/kg x 4 days; 60 mg/kg total dose) along with methotrexate (MTX) and cyclosporine (CSA) immunosuppression to prevent GvHD. The five-year survival rate for all patients was approximately 85%, and 96% among the 48 patients who were younger than 10 years at the time of HCT.

T-Cell Depletion Reduces Risk of Graft-Versus-Host Disease

The Minnesota group reported that T-cell depletion of bone marrow in sibling donors reduced the risk of GvHD [19]. In this report, patients were conditioned with CY (5 mg/kg x 4 days; 20 mg/kg total dose), FLU (35 mg/m^2 x 5 days; 175 mg/m^2 total dose), and anti-thymocyte globulin (ATG) (30 mg/kg x 5 days; 150 mg/kg total dose) followed by the infusion of T-cell-depleted marrow with CSA and either methylprednisolone or mycophenolate mofetil (MMF) to prevent GvHD. Of the 23 patients (median age 8.5 years; ranging from 3.2 - 43.3 years) included in the study, 92% survived at least five years [19]. These results indicate that when possible, T-cell depletion should be used to reduce the risk of GvHD.

Unrelated Donor Hematopoietic Cell Transplant

The majority of FA patients do not have an HLA-identical unaffected sibling donor, so alternative types of donors must be explored. The two most common donor types are adult volunteers registered with organizations like the National Marrow Donor Program (NMDP) and unrelated cord blood (UCB) obtained from the placenta after the birth of a baby.

Recently, the use of haploidentical donors also has been explored as an alternative donor source.

Outcomes of HCT were reported by the Minnesota group on 48 patients with FA (ranging from 1.7-34.3 years) with aplastic anemia or MDS who received FLU, CY, ATG, and low-dose TBI (300 cGy) followed by T-cell-depleted 7-8/8 HLA-matched bone marrow (32 patients) or by HLA-mismatched UCB (16 patients) if an unrelated donor was unavailable [20]. In this study, recipients of bone marrow engrafted at a median of 11 days (ranging from 9-23 days); in contrast, only 88% of cord blood recipients engrafted at a median of 19 days (ranging from 10-40 days). Incidence of acute and chronic GvHD was low (12% and 6%, respectively), with similar outcomes in patients transplanted with bone marrow and UCB. The overall survival for the entire cohort was 78% at a median of 2.9 years (ranging from 0.6-6.3 years). However, patients without a prior history of opportunistic infection or transfusions had a 92% chance of survival at five years [20].

In 2017, the Cincinnati group reported results of a multi-institutional study in which a radiation-free conditioning regimen was tested, replacing TBI with busulfan (BU) for patients undergoing alternative donor HCT, including haploidentical transplants. Forty-five patients (median age 8.2 years, range 4.3-44 years), with aplastic anemia and/or MDS, received FLU, CY, and ATG in combination with BU followed by transplantation of T-cell-depleted peripheral blood stem cells. All but one patient engrafted at a median of nine days (range 7-15). The incidence of acute GvHD was low at 7%. Three patients developed limited chronic GvHD and none developed extensive chronic GvHD. The median follow-up time was 41 months and three-year overall survival was 80%. All 19 patients younger than 10 years of age who were transplanted for severe marrow failure and with lower dose BU survived [21]. This group of investigators is now testing a risk-adjusted approach to the use of radiation-free transplant by giving lower doses of BU to those with aplasia and higher doses to those with MDS or AML.

The costs of unrelated donor and cord blood HCT are prohibitive in many countries; therefore, the use of haploidentical donors has been explored as an alternative donor option. Outcomes have improved dramatically for alternative donor HCT with the use of post-HCT (PT) cyclophosphamide (CY) for GvHD prophylaxis, a strategy that raises specific challenges in CY-sensitive FA patients. In 2017, Bonfim et al. reported on the results of this approach in 30 patients with FA after a preparative regimen of FLU, TBI (200-300 cGy), and CY with or without ATG [22]. All patients received PT-CY (25 mg/kg/d x 2 doses) followed by CSA and MMF. All patients engrafted in the subgroup of patients who did not receive ATG (n = 14), but their HCT course was complicated by high rates of acute and chronic GvHD, and only 8 patients survived at time of reporting. In the subgroup that received ATG (n = 16), 14 patients had sustained engraftment, severe GvHD rates were lower, and 13 patients are alive. One-year overall survival for the entire cohort was 73%. These data demonstrate that haploidentical donor transplantation with PT-CY is feasible for FA patients without a matched related or unrelated donor; however, haploidentical

transplants still offer challenges [22] and should be considered in patients with FA only if there are no other alternatives.

In 2018, the Minnesota group compared outcomes of HLA-matched sibling donor (MSD) HCT (n = 17) and alternative donor HCT (n = 57) performed in patients with FA who had severe aplastic anemia between 2001 and 2016 [23]. Overall survival at five years was 94% for MSD-HCT versus 86% for alternative donor-HCT; neutrophil engraftment was 100% versus 95%, and platelet recovery was 100% versus 89%. Acute GvHD was 6% versus 12%, severe acute GvHD was 6% versus 4%, and chronic GvHD was 0% versus 7%, with no statistically significant differences noted by the type of transplant. These data demonstrate that alternative donor-HCT should be considered when a patient is close to transfusion-dependence, similar to timing for MSD-HCT, in patients with FA-associated BMF.

Indications for Sibling and/or Alternative Donor Hematopoietic Cell Transplant

The eligibility criteria to consider for sibling or alternative donor HCT is as follows:

- Aplastic anemia (hemoglobin (Hgb) < 8 g/dL or absolute neutrophil count (ANC) < 500/μL or platelet count < 20,000/μL)
- MDS or AML
- Progressive complex cytogenetic abnormalities known to be associated with malignancy
- Absence of active infections
- Available donor
  Order of priority
  - HLA 10/10 (followed by 9/10) allele-matched sibling
  - HLA 10/10 (followed by 9/10) allele-matched relative other than sibling
  - HLA 10/10 (followed by 9/10) allele-matched unrelated adult volunteer
  - HLA 10-8/10 antigen matched UCB

Indications for Hematopoietic Cell Transplant

With similar outcomes, the indications for alternate donor HCT are the same as the indications for sibling donor HCT. Patients with an exceptional risk of HCT-related mortality (e.g., patients with severe organ dysfunction, those who are 35 years or older, and those with pre-existing malignancies or life-threatening systemic infections) may consider alternative treatment options first, such as the use of androgens.

Patients with FA who develop persistent and severe cytopenias or evidence of MDS or AML should be considered for HCT provided the patient is not too old and has adequate organ function. Clinical investigation is underway to determine whether HCT performed earlier may be considered for patients with specific genetic mutations who are deemed to be at particularly high risk for rapid progression to MDS or AML, and who may face markedly shortened survival times (e.g., *BRCA*-related genetic mutations) [24].

Graft-Versus-Host Disease

Graft-versus-host disease (GvHD) occurs when the transplanted immune system of the donor recognizes the patient as "foreign" and tries to reject the foreign tissues. This disease sometimes occurs after HCT because the donor's immune system is transplanted along with the donor's hematopoietic stem cells (HSC), which are responsible for marrow recovery and reconstitution of the blood cells. While GvHD can occur in any patient undergoing an allogeneic HCT, the disease tends to be more common and severe in mismatched donor recipients. The signs and symptoms of the two types of GvHD (acute and chronic) are detailed in Table 2. Graft-versus-host disease can occur regardless of the prophylactic approach used. The more severe the GvHD (e.g., grade 3-4 disease), the higher the risk of death, mostly due to infection. If GvHD occurs, the typical first line treatment is a steroid (methylprednisolone).

Table 2. Signs and symptoms of acute and chronic GvHD.

Graft-versus-host disease (GvHD)	
Acute GvHD	**Chronic GvHD**
Skin rash (blistering with more severe disease) Persistent nausea Diarrhea Jaundice	Skin rash, discoloration Hair loss Dry mouth, tooth decay Dry eyes Sores in the mouth, thrush Ridged or fragile nails Shortness of breath, exercise intolerance Anorexia, weight loss Stiff joints

Infections

Infection following HCT can be a major complication for FA patients due to their unique sensitivity to chemoradiotherapy and, in some cases, the extensive period of neutropenia prior to HCT for some patients. Prophylactic antibiotic regimens are commonly used after HCT to reduce the risk of infection. Most patients are treated with trimethoprim/sulfamethoxazole (Bactrim) for one year after transplant and other antibacterial and antifungal drugs through at least day 100 after HCT. The length of prophylactic therapy to prevent infection depends upon the degree of immunosuppression, the patient's absolute CD4 T-cell level, the development of acute or chronic GvHD, and the patient's prior history of infectious complications.

Long-Term Follow-Up Care for Hematopoietic Cell Transplant

Long-term follow-up after HCT for patients with FA must be thought of as an indispensable part of routine medical care. Guidelines for the long-term care of survivors of childhood cancer have been developed by the Children's Oncology Group [25]. In addition, the American Society of Blood and Marrow Transplantation, the European Group for Blood and Marrow Transplantation, and the Center for International Blood and Marrow Transplant Research recently developed joint recommendations [26] which include suggested screening and preventive practices for adult survivors of HCT. Many of these recommendations also apply to patients with FA who have undergone HCT.

All patients treated with HCT, including those with FA, are subject to health complications known as "late effects" that may develop long after the transplant. These effects include late graft failure, recurrent acute and chronic GvHD, and the effects of prolonged steroid therapy such as hypertension, hyperglycemia, and aseptic necrosis of bone (loss of bone primarily in the hip, knee, and shoulder joints). Other HCT late effects such as short stature and sterility have not been formally evaluated in patients with FA since these are pre-existing problems in most FA patients. Late effects of transplant can negatively impact the patient's physical and mental health, quality of life, growth, development, education, and employment (Table 3). Therefore, the development of late effects must be assessed on an ongoing basis [27-36].

Table 3. Possible long-term adverse effects and their causes in patients with FA.

Organ or system affected	Adverse effects	Causes
General	Short stature	FA, HCT
	Primary or secondary cancers	FA, HCT, GvHD
Skin	Pigmentation	FA, GvHD
	Dryness	FA, GvHD
	Thickening	FA, GvHD
Central nervous system	Side effects of radiation	HCT
Eyes	Cataracts	HCT
	Very dry eyes (Sicca, or Sjögren's syndrome)	GvHD
	Retinitis	HCT
Ears, nose, and throat	Chronic sinusitis	GvHD
	Hearing loss	FA, HCT
	Very dry mouth (Sicca, or Sjögren's syndrome)	GvHD

Organ or system affected	Adverse effects	Causes
Heart	Congenital anomalies	FA
	Iron overload	Blood transfusions
Lungs	Side effects of HCT	HCT, GvHD
Liver	Chronic liver disease (transaminitis or cholestasis)	HCT, GvHD
	Iron overload	FA or HCT treatment (transfusions)
Kidneys and genitourinary system	Congenital anomalies	FA
	Chronic renal insufficiency	HCT
GI tract	Congenital anomalies	FA
	Failure to thrive	FA, GvHD
	Functional problems (e.g., malabsorption)	FA, GvHD
Endocrine	Diabetes	FA, GvHD
	Hypothyroidism	FA, HCT
Gonadal	Masculinization (virilization)	Androgens
	Infertility	FA, HCT
	Early menopause	FA, HCT
Musculoskeletal	Hand and arm anomalies	FA
	Hip dysplasia	FA
Psychological	Psychosocial issues (e.g., anxiety, depression)	FA, HCT, GvHD

Practical Considerations for Long-Term Follow-Up Care

General guidelines for long-term follow-up of FA patients starting at one year post-HCT is outlined in Table 4 [27, 37-39]. Long-term care plans should be tailored to the specific needs of each individual FA patient under the supervision of a long-term care team comprised of the HCT physician, primary hematologist, and a multi-disciplinary team of specialists.

Table 4. General post-HCT long-term follow-up guidelines for patients with FA.

	1 year	2 year	3 year	4 year	5 year	Yearly
Regular check-ups, including patient history and physical exam	X	X	X	X	X	X
HEMATOLOGY						
Complete blood counts	X	X	X	X	X	X

	1 year	2 year	3 year	4 year	5 year	Yearly
Bone marrow aspiration Chimerism testing Cytogenetics studies	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	
Measure levels of ferritin and iron Perform T2*MRI if ferritin levels are high	If clinically indicated	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	
IMMUNOLOGY						
Assess immune phenotype and function	X	Repeat if previous test was abnormal	Repeat if previous test was abnormal			
Measure levels of immunoglobulins G, A, and M	X	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	
Administer immunizations (including HPV vaccine)	X	As per schedule				Administer boosters as needed
CARDIAC						
Measure fasting lipid profile (levels of total cholesterol, LDL, HDL, and triglycerides)	X	Repeat if previous test was abnormal	X	Repeat if previous test was abnormal	X	Repeat if previous test was normal
EKG	X	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	X	Repeat if previous test was normal
Echocardiogram	X	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	X	Repeat if previous test was normal
PULMONARY						
Perform pulmonary function testing to rule out obstructive or restrictive disease	X	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	X	
HEPATIC						
Measure liver function panel	X	X	X	X	X	X
If liver function panel values are high, consider the need for liver biopsy	Only if previous test was abnormal					
Measure levels of ferritin and iron Perform T2*MRI if ferritin levels are high	If clinically indicated	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	Repeat if previous test was abnormal	

	1 year	2 year	3 year	4 year	5 year	Yearly
RENAL						
Measure levels of electrolytes, BUN, and creatinine in the urine	X	X	X	X	X	X
Perform urinalysis	X		X		X	
ENDOCRINE and METABOLISM						
Perform an oral glucose tolerance test (OGTT)	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated
Measure levels of TSH and FT4	X	X	X	X	X	X
Measure levels of FSH and LH in patients younger than 10 years Measure estradiol levels in female patients older than 10 years Measure testosterone levels in male patients older than 11 years	X	X	X	X	X	As needed
Measure levels of IGF-1 and IGFBP-3 in patients younger than 18 years	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	
Measure levels of 25-OH vitamin D and calcium	X	X	X	X	X	X
Assess bone age in patients 5 to 18 years	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	
DXA scan (with adjustment for height)	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated
GROWTH and DEVELOPMENT						
Plot patient's height and weight on a growth chart	X	X	X	X	X	X
Neuropsychological evaluation	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	
HEAD and NECK						
Ophthalmology evaluation	X	If clinically indicated	If clinically indicated	If clinically indicated	If clinically indicated	As needed
Screen for head and neck cancers (performed by a head and neck specialist)	Every 6 months	Every 6 months	Every 6 months	Every 6 months	Every 6 months	Every 6 months
Hearing evaluation	X		As needed		As needed	
Biannual dental evaluations	Every 6 months	Every 6 months	Every 6 months	Every 6 months	Every 6 months	Every 6 months

	1 year	2 year	3 year	4 year	5 year	Yearly
GYNECOLOGIC						
General gynecologic evaluation and cancer screening in female patients older than 13 years	X	X	X	X	X	X
DERMATOLOGY						
Evaluate nevi and check for skin cancers	X	X	X	X	X	X
Testing to rule out GvHD of skin	X	X	X	X	X	X

Alternative Treatments for Bone Marrow Failure

If blood counts decline to severe levels (Table 1) and cure by HCT is not possible or preferred, alternative therapies may maintain blood counts and quality of life in patients with FA.

Androgens

Synthetic androgens, such as oxymetholone and danazol, have been used to treat cytopenias in patients with FA for more than 50 years. Androgens primarily affect red cells and platelets but can also improve neutrophil counts [40, 41]. More than half of patients with FA who are treated with androgens will respond at least transiently, although a subset of patients who initially respond may become refractory over time. As many as 10-20% of patients who receive continuous low dose androgen therapy may never require an HCT, unless MDS and/or AML develop. Thus, androgen treatment may delay a transplant for months and even years in responsive patients [42].

Androgen treatment used to delay HCT may be associated with the following risks and complications:

- Androgens do not prevent progression to MDS/AML
- Androgens may increase the chance of liver or other problems, which may complicate HCT
- Use of androgens may increase the age at which patients with FA undergo HCT
- Patients may have acquired viral infections, which may be problematic during HCT

Patients prescribed androgens should be prospectively monitored for liver function test (LFT) abnormalities and development of liver tumors. Blood LFTs should be performed every 3-6 months, and a liver ultrasound should be performed every 6-12 months. If the

levels of liver transaminases increase to 3-5 times above normal, the androgen dose should be tapered until the blood tests improve. Androgen-associated liver adenomas (benign tumors) may develop with long-term treatment and are predominantly due to the cellular liver toxicities of the 17 alpha-alkylated androgens. Liver adenomas may resolve after androgens are discontinued, but some may persist for years after androgen therapy has ended. Liver adenomas are not a contraindication for HCT. If screening tests raise a concern for hepatocellular carcinoma, a liver biopsy using a technique appropriate to the patient's bleeding risk should be considered. Even without additional risk factors, malignant transformation of initially benign hepatic adenomas may occur after years of androgen treatment [43].

Oxymetholone

The most commonly used androgen since 1961 is oxymetholone [40, 41]. The starting dose of oxymetholone is typically ~2 mg/kg/day, but doses as high as 5 mg/kg/day may be required. Most patients who will respond do so within three to four months with stabilization of falling counts or an increase in the hemoglobin or platelet counts. If a response occurs, then the general strategy is to slowly taper the daily dose of oxymetholone in 10-20% decrements every three to four months until the lowest effective dose with minimal side effects is obtained. Over time, the side effects of accelerated linear growth (ultimately with premature closure of the growth plates) and weight gain effectively reduce the individual's dose per kilogram body weight; therefore, the patient's dose per kilogram body weight should be recalculated prior to making dose adjustments.

The patient (both male and female) and family should be counseled about the possible side effects of oxymetholone. Every effort should be made to minimize the side effects by tapering the dose to the minimum effective dose whenever possible. Aggressive acne treatment of facial and back lesions may make the treatment more tolerable. Discussion of the masculinizing side effects, such as hair darkening and development on lip/groin/axilla and deepening of the voice, should occur before prescription. Long-term androgen usage may lead to shrinkage and/or impaired development of the testis in males due to suppression of the hypothalamic-pituitary-gonadal axis.

If no response is seen after three to four months, then—in the absence of other causes of cytopenias such as viral, bacterial, or fungal infection—oxymetholone should be discontinued (although there are anecdotal reports of rare patients responding after six or more months). Stabilization of hemoglobin levels may be seen sooner than improvements in platelet counts; white cell responses may occur later or be nonexistent.

Danazol

A few reports [44-46] in the literature show that both male and female FA patients may benefit from treatment with danazol, an attenuated synthetic androgen that produces fewer virializing effects than oxymetholone and may cause fewer liver complications. A recent retrospective study demonstrated the effectiveness of danazol in 7 of 8 patients

with FA (starting dose 3.5-7.7 mg/kg/day), including 3 patients (2 females and 1 male) who were treated successfully for more than three years and 1 patient (female) for more than 10 years without exhibiting progressive marrow failure requiring stem cell transplantation [46]. The comparative efficacy of danazol versus oxymetholone to treat marrow failure in patients with FA is unknown. Danazol has been used at doses of 200-800 mg/day (3.3-13.3 mg/kg/day for a 60 kg woman) for months in women to treat endometriosis and is still used as long-term prophylaxis for hereditary angioedema at a dose of approximately 5 mg/kg/day [47].

Metformin

Metformin is a drug approved by the U.S. Food and Drug Administration for treatment of diabetes mellitus that has shown promise in treating hematologic issues in preclinical FA models. In these studies, metformin increased blood counts and protected cells against DNA damage [48, 49]. Researchers at Harvard University initiated a phase I clinical trial in 2017 to explore whether metformin increases blood counts in patients with FA. As of June 2020, the trial was still recruiting patients and results from the trial had not yet been published.

Quercetin

The University of Cincinnati initiated a phase 1 clinical trial in 2012 to assess the safety profile of oral quercetin therapy in patients with FA. Studies have shown that systemic reactive oxygen species (ROS) contribute to hematopoietic progenitor cells fragility [50]. Quercetin, a naturally occurring flavonoid found in fruits and vegetables, scavenges free radicals and has anti-inflammatory, antioxidant and antineoplastic properties [51, 52]. The goal of the phase 1 pilot study was to determine long-term safety and efficacy of quercetin administration in patients with FA. Secondary endpoints included identifying the effects of quercetin on blood counts. Dose optimization from the phase 1 trial led to development of a phase II quercetin chemoprevention trial that was initiated in 2018 by the Cincinnati group. The goal of the phase II trial is to determine the efficacy of a maximum daily dose of quercetin (4,000 mg/day) in reducing buccal micronuclei as a surrogate marker for DNA damage and susceptibility to squamous cell carcinoma in FA patients post-HCT. As of June 2020, the phase II trial was still recruiting patients and results from the trial had not yet been published.

Cytokines

Several cytokines have been evaluated for their capacity to stimulate failing bone marrow in FA patients, but none has proven entirely successful. The cytokines granulocyte colony-stimulating factor (G-CSF) [53] and granulocyte-macrophage colony-stimulating factor (GM-CSF) [54] can improve the neutrophil count in patients with FA; however, GM-CSF is no longer available for clinical use. Treatment with other cytokines has not shown benefit for

patients with FA. However, newer agents such as thrombopoietin-mimetic drugs are being cautiously tested in patients with FA [55].

Treatment with G-CSF may be considered if the neutropenia is associated with recurrent or serious infections, particularly if the neutrophil count is persistently below 500/mm^3 or as a short-term bridge to transplant. There is, however, concern that cytokine therapy will stimulate development or progression of cytogenetic abnormalities. Historically, a few patients also have shown improvements in hemoglobin levels or platelet counts while on G-CSF; these effects most likely are due to the treatment of, or reduction in, infections. Long-term follow-up has not been published. Treatment should generally be discontinued if the neutrophil count fails to improve after eight weeks of G-CSF therapy.

A bone marrow aspirate or biopsy with cytogenetics is recommended prior to the initiation of cytokine treatment, given the risk of stimulating the growth of a leukemic clone. It is recommended that patients being treated with cytokines are monitored for bone marrow morphology and cytogenetics every six months. In the setting of a compelling clinical indication for cytokine therapy, such as an acute infection, there are no findings to support withholding cytokines from patients with clonal abnormalities. In such cases, the use of hematopoietic cytokines should be considered only in consultation with experts in the care of patients with FA.

Transfusion of Blood Products

Transfusions of red cells or platelets may be needed prior to surgery in patients with the following:

- Anemia and/or thrombocytopenia
- Progressive marrow failure
- Bone marrow failure that precludes all prospect of an early HCT (due to the lack of an acceptable donor, severe organ dysfunction, comorbidities, socioeconomic situations, and/or lack of interest in pursuing HCT as a therapy)

Long-term transfusions with red cells and platelets may become a lifeline for patients for whom no other treatment options are available. However, if HCT is the goal, transfusions should be minimized.

Gene Therapy

Gene therapy has been employed for multiple conditions with a hemopoietic component, including hemoglobinopathies [56], leukemia [57], immunodeficiencies [58], lysosomal storage disease [59], and Fanconi anemia. The first clinical trials of stem cell gene therapy for FA used retroviruses to deliver the *FANCA* or *FANCC* genes. This early protocol, however, resulted in either no correction or only transient correction of hematopoietic cells, an observation consistent with only short-term functional gene complementation [60-63].

Lessons learned from earlier gene therapy clinical trials and preclinical animal model studies [64-67] cumulatively led to development of improved clinical trial protocols. The first successful gene therapy trial for patients with variants in *FANCA* demonstrated that lentiviral-mediated hematopoietic gene transfer into hematopoietic stem cells followed by delivery in non-conditioned patients led to successful engraftment and expansion of *FANCA* gene-corrected cells [68]. Functional laboratory studies also demonstrated that the normal cells expressed a functional *FANCA* protein, as the cells were resistant to DNA damaging agents. Importantly, no adverse events have been reported in any of the patients to date in the ongoing study. Additional clinical gene therapy trials for patients with *FANCA* variants also have been initiated. These trials are addressing additional challenges with FA gene therapy, such as the role of conditioning and optimization of the ex vivo hematopoietic stem cell culture.

Gene Editing

Gene editing also is on the horizon as a useful therapy to treat bone marrow failure in patients with FA, but research is currently in pre-clinical stages. A key differentiating feature between gene editing and gene therapy is precise gene modification. Current gene editing systems include zinc finger nucleases (ZFN), meganucleases (MN), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 system. Each system is unique, but each shares the function that they bind DNA and generate a break in one or both DNA strands. Following this break, the DNA can be repaired through an error-prone process where the DNA ends are reconnected. To date, ZFNs, TALENs, and CRISPR/Cas9 have been used in FA gene modification in the laboratory [69, 70]; however, because gene editing causes DNA breaks and FA proteins are required to repair DNA breaks [71] this method may not be viable for FA patients. More pre-clinical research is needed to determine whether gene editing will be efficacious for patients with FA.

Summary

Fanconi anemia (FA) is a genetic disorder that results in DNA repair defects that adversely affect the stability of hematopoietic stem cells (HSCs). This results in the high likelihood that patients with FA will develop bone marrow failure (BMF) and/or clonal progression to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). The only cure for BMF at the present time is a hematopoietic cell transplant (HCT). Recommendations for clinical monitoring of BMF are based on the stability of peripheral blood counts and clonal abnormalities observed in serial bone marrow examinations. The decision to use HCT for FA patients with BMF and/or clonal abnormalities, MDS, or leukemia should be made in consultation with an FA physician specialist. Recent advancements in HCT protocols continue to improve survival rates. These advancements include, but are not limited to, knowledge that HCT without total body radiation is successful, T-cell depletion should be used when possible, and that HCT from mis-matched related, unrelated and haploidentical donors can be successful for patients without any other donor options. Transplants in general, because of graft-versus-host disease, the conditioning regimens, and long periods of immunosuppression, confer an increased risk of earlier onset cancer. This indicates that close follow up during long-term care following HCT is imperative. Emerging therapies such as gene therapy also hold promise as curative options for BMF in patients with FA and the future of BMF treatment for patients with FA will undoubtedly shift as gene therapy and gene editing technology matures and efficacy is established.

The Fanconi Anemia Research Fund recognizes the following author contributions to the 5th edition:

Bone marrow failure section:
Zora R. Rogers, MD*

Hematopoietic cell transplant section:
Margaret L. MacMillan, MD*
Stella Davies, MBBS, PhD, MCRP
John E. Wagner, MD

Long-term follow up section:

Eva Guinan, MD*
Farid Boulad, MD
Maria Cancio, MD
Stella Davies, MBBS, PhD, MCRP

Gene therapy section:
Mark J. Osborn, PhD*
Christen L. Ebens, MD, MPH

**Section Committee Chair*

References

1. Auerbach, A.D., *Fanconi anemia and its diagnosis.* Mutat Res, 2009. 668(1-2): p. 4-10.
2. Parikh, S. and M. Bessler, *Recent insights into inherited bone marrow failure syndromes.* Curr Opin Pediatr, 2012. 24(1): p. 23-32.
3. Shimamura, A. and B.P. Alter, *Pathophysiology and management of inherited bone marrow failure syndromes.* Blood Rev, 2010. 24(3): p. 101-22.
4. Alter, B.P., *Cancer in Fanconi anemia, 1927-2001.* Cancer, 2003. 97(2): p. 425-40.
5. Kutler, D.I., et al., *A 20-year perspective on the International Fanconi Anemia Registry (IFAR).* Blood, 2003. 101(4): p. 1249-56.
6. Rosenberg, P.S., M.H. Greene, and B.P. Alter, *Cancer incidence in persons with Fanconi anemia.* Blood, 2003. 101(3): p. 822-6.
7. Huck, K., et al., *Delayed diagnosis and complications of Fanconi anaemia at advanced age--a paradigm.* Br J Haematol, 2006. 133(2): p. 188-97.
8. Butturini, A., et al. *Hematologic abnormalities in Fanconi anemia: an International Fanconi anemia registry study.* Blood, 1994. 85 (5): p. 1650-55.
9. Alter, B.P., et al., *Fanconi anemia: myelodysplasia as a predictor of outcome.* Cancer Genet Cytogenet, 2000. 117(2): p. 125-31.

10. Mehta, P.A., et al., *Numerical chromosomal changes and risk of development of myelodysplastic syndrome--acute myeloid leukemia in patients with Fanconi anemia.* Cancer Genet Cytogenet, 2010. 203(2): p. 180-6.

11. Meyer, S., H. Neitzel, and H. Tonnies, *Chromosomal aberrations associated with clonal evolution and leukemic transformation in fanconi anemia: clinical and biological implications.* Anemia, 2012. 2012: p. 349837.

12. Rochowski, A., et al., *Patients with Fanconi anemia and AML have different cytogenetic clones than de novo cases of AML.* Pediatr Blood Cancer, 2012. 59(5): p. 922-4.

13. Tonnies, H., et al., *Clonal chromosomal aberrations in bone marrow cells of Fanconi anemia patients: gains of the chromosomal segment 3q26q29 as an adverse risk factor.* Blood, 2003. 101(10): p. 3872-4.

14. Cioc, A.M., et al., *Diagnosis of myelodysplastic syndrome among a cohort of 119 patients with fanconi anemia: morphologic and cytogenetic characteristics.* Am J Clin Pathol, 2010. 133(1): p. 92-100.

15. Vundinti, B.R., S. Korgaonkar, and K. Ghosh, *Incidence of malignancy and clonal chromosomal abnormalities in Fanconi anemia.* Indian J Cancer, 2010. 47(4): p. 397-9.

16. Mitchell, R., et al., *Haematopoietic cell transplantation for acute leukaemia and advanced myelodysplastic syndrome in Fanconi anaemia.* Br J Haematol, 2014. 164(3): p. 384-95.

17. Wagner, J.E., et al., *Hematopoietic cell transplantation for Fanconi anemia,* in *Thomas' Hematopoietic Cell Transplantation, 5th ed*, K.G. Blume, S.J. Forman, and F.R. Appelbaum, Editors. 2013, Blackwell Publishing, Ltd: Oxford.

18. Bonfim, C., *HLA-matched related bone marrow transplantation in 85 patients with Fanconi anemia: The Brazilian experience using cyclophosphamide 60 mg/kg.* Biol Blood Marrow Transplant, 2012. 18(2): p. S209.

19. Tan, P.L., et al., *Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation.* Pediatr Blood Cancer, 2006. 46(5): p. 630-6.

20. MacMillan, M.L., et al., *Alternative donor hematopoietic cell transplantation for Fanconi anemia.* Blood, 2015. 125(24): p. 3798-804.

21. Mehta, P.A., et al., *Radiation-free, alternative-donor HCT for Fanconi anemia patients: results from a prospective multi-institutional study.* Blood, 2017. 129(16): p. 2308-15.

22. Bonfim, C., et al., *Haploidentical bone marrow rransplantation with post-transplant cyclophosphamide for children and adolescents with Fanconi anemia.* Biol Blood Marrow Transplant, 2017. 23(2): p. 310-17.

23. Ebens, C.L., et al., *Comparable outcomes after HLA-matched sibling and alternative donor hematopoietic cell transplantation for children with Fanconi anemia and severe aplastic anemia.* Biol Blood Marrow Transplant, 2018. 24(4): p. 765-71.

24. Wagner, J.E., et al., *Germline mutations in BRCA2: shared genetic susceptibility to breast cancer, early onset leukemia, and Fanconi anemia.* Blood, 2004. 103(8): p. 3226-9.

25. Children's Oncology Group, *Long-term follow-up guidelines for children's cancer survivors.* 2020. Accessed: http://www.survivorshipguidelines.org

26. Majhail, N.S., et al., *Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation.* Biol Blood Marrow Transplant, 2012. 18: p. 348-71.

27. Anur, P., et al., *Late effects in patients with Fanconi anemia following allogeneic hematopoietic stem cell transplantation from alternative donors*. Bone Marrow Transplant, 2016. 51(7): p. 938-44.

28. Armenian, S.H., et al., *Children's Oncology Group's 2013 blueprint for research: survivorship and outcomes*. Pediatr Blood Cancer, 2013. 60(6): p. 1063-8.

29. Baker, K.S., et al., *Diabetes, hypertension, and cardiovascular events in survivors of hematopoietic cell transplantation: a report from the bone marrow transplantation survivor study*. Blood, 2007. 109(4): p. 1765-72.

30. Boulad, F., S. Sands, and C. Sklar, *Late complications after bone marrow transplantation in children and adolescents*. Curr Probl Pediatr, 1998. 28(9): p. 273-97.

31. Filipovich, A.H., et al., *National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report*. Biol Blood Marrow Transplant, 2005. 11(12): p. 945-56.

32. Leiper, A.D., *Non-endocrine late complications of bone marrow transplantation in childhood: part II*. Br J Haematol, 2002. 118(1): p. 23-43.

33. Leiper, A.D., *Non-endocrine late complications of bone marrow transplantation in childhood: part I*. Br J Haematol, 2002. 118(1): p. 3-22.

34. Pulsipher, M.A., et al., *National Cancer Institute, National Heart, Lung and Blood Institute/Pediatric Blood and Marrow Transplantation Consortium First International Consensus Conference on late effects after pediatric hematopoietic cell transplantation: the need for pediatric-specific long-term follow-up guidelines*. Biol Blood Marrow Transplant, 2012. 18(3): p. 334-47.

35. Rizzo, J.D., et al., *Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation: joint recommendations of the European Group for Blood and Marrow Transplantation, the Center for International Blood and Marrow Transplant Research, and the American Society of Blood and Marrow Transplantation*. Biol Blood Marrow Transplant, 2006. 12(2): p. 138-51.

36. Socie, G., et al., *Nonmalignant late effects after allogeneic stem cell transplantation*. Blood, 2003. 101(9): p. 3373-85.

37. Bonfim, C., et al., *Long-term survival, organ function, and malignancy after hematopoietic stem cell transplantation for Fanconi anemia*. Biol Blood Marrow Transplant, 2016. 22(7): p. 1257-63.

38. Dietz, A.C., et al., *Current knowledge and priorities for future research in late effects after hematopoietic cell rransplantation for inherited bone marrow failure syndromes: consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric hematopoietic cell transplantation*. Biol Blood Marrow Transplant, 2017. 23(5): p. 726-35.

39. Dietz, A.C., et al., *Late effects screening guidelines after hematopoietic cell transplantation for inherited bone marrow failure syndromes: consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric HCT*. Biol Blood Marrow Transplant, 2017. 23(9): p. 1422-28.

40. Diamond, L.K. and N.T. Shahidi, *Treatment of aplastic anemia in children*. Semin Hematol, 1967. 4(3): p. 278-88.

41. Shahidi, N.T. and L.K. Diamond, *Testosterone-induced remission in aplastic anemia of both acquired and congenital types. Further observations in 24 cases*. N Engl J Med, 1961. 264: p. 953-67.

42. Paustian, L., et al., *Androgen therapy in Fanconi anemia: A retrospective analysis of 30 years in Germany*. Pediatr Hematol Oncol, 2016. 33(1): p. 5-12.

43. Velazquez, I. and B.P. Alter, *Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions*. Am J Hematol, 2004. 77(3): p. 257-67.

44. Basu, S., et al., *Fanconi anemia*. Indian J Pediatr, 1996. 63(3): p. 399-402.

45. Zatterale, A., et al., *Identification and treatment of late onset Fanconi's anemia*. Haematologica, 1995. 80(6): p. 535-8.

46. Scheckenbach, K., et al., *Treatment of the bone marrow failure in Fanconi anemia patients with danazol*. Blood Cells Mol Dis, 2012. 48(2): p. 128-31.

47. Zuraw, B.L., *Clinical practice. Hereditary angioedema*. N Engl J Med, 2008. 359(10): p. 1027-36.

48. Crossan, G.P., *Metformin: treating the cause of Fanconi anemia?* Blood, 2016. 128(24): p. 2748-50.

49. Zhang, Q.S., et al., *Metformin improves defective hematopoiesis and delays tumor formation in Fanconi anemia mice*. Blood, 2016. 128(24): p. 2774-84.

50. Sejas, D.P., et al., *Inflammatory reactive oxygen species-mediated hemopoietic suppression in Fancc-deficient mice*. J Immunol, 2007. 178(8): p. 5277-87.

51. Chen, S.F., et al., *Reappraisal of the anticancer efficacy of quercetin in oral cancer cells*. J Chin Med Assoc, 2013. 76(3): p. 146-52.

52. Kashyap, D., et al., *Fisetin and quercetin: promising flavonoids with chemopreventive potential*. Biomolecules, 2019. 9(5).

53. Rackoff, W.R., et al., *Prolonged administration of granulocyte colony-stimulating factor (filgrastim) to patients with Fanconi anemia: a pilot study*. Blood, 1996. 88(5): p. 1588-93.

54. Guinan, E.C., et al., *Evaluation of granulocyte-macrophage colony-stimulating factor for treatment of pancytopenia in children with fanconi anemia*. J Pediatr, 1994. 124(1): p. 144-50.

55. Ecsedi, M., et al., *Use of eltrombopag in aplastic anemia in Europe*. Ann Hematol, 2019. 98(6): p. 1341-50.

56. Thompson, A.A., et al., *Gene therapy in patients with transfusion-dependent beta-thalassemia*. N Engl J Med, 2018. 378(16): p. 1479-93.

57. Maude, S.L., et al., *Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia*. New England Journal of Medicine, 2018. 378(5): p. 439-48.

58. Mamcarz, E., et al., *Lentiviral gene therapy combined with low-dose busulfan in infants with SCID-X1*. N Engl J Med, 2019. 380(16): p. 1525-34.

59. Biffi, A., et al., *Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy*. Science, 2013. 341(6148): p. 1233158.

60. Williams, D.A., J. Croop, and P. Kelly, *Gene therapy in the treatment of Fanconi anemia, a progressive bone marrow failure syndrome*. Curr Opin Mol Ther, 2005. 7(5): p. 461-6.

61. Liu, J.M., et al., *Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene (FANCC)*. Hum Gene Ther, 1999. 10(14): p. 2337-46.

62. Walsh, C.E., et al., *A functionally active retrovirus vector for gene therapy in Fanconi anemia group C*. Blood, 1994. 84(2): p. 453-9.

63. Muller, L.U. and D.A. Williams, *Finding the needle in the hay stack: hematopoietic stem cells in Fanconi anemia*. Mutat Res, 2009. 668(1-2): p. 141-9.

64. Galimi, F., et al., *Gene therapy of Fanconi anemia: preclinical efficacy using lentiviral vectors*. Blood, 2002. 100(8): p. 2732-6.

65. Molina-Estevez, F.J., et al., *Lentiviral-mediated gene therapy in Fanconi anemia-A mice reveals long-term engraftment and continuous turnover of corrected HSCs*. Curr Gene Ther, 2015. 15(6): p. 550-62.

66. Muller, L.U., et al., *Rapid lentiviral transduction preserves the engraftment potential of Fanca(-/-) hematopoietic stem cells*. Mol Ther, 2008. 16(6): p. 1154-60.

67. Rio, P., et al., *In vitro phenotypic correction of hematopoietic progenitors from Fanconi anemia group A knockout mice*. Blood, 2002. 100(6): p. 2032-9.

68. Rio, P., et al., *Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia*. Nat Med, 2019. 25(9): p. 1396-1401.

69. Rio, P., et al., *Targeted gene therapy and cell reprogramming in Fanconi anemia*. EMBO Mol Med, 2014. 6(6): p. 835-48.

70. Osborn, M.J., et al., *Fanconi anemia gene editing by the CRISPR/Cas9 system*. Hum Gene Ther, 2015. 26(2): p. 114-26.

71. Richardson, C.D., et al., *CRISPR-Cas9 genome editing in human cells occurs via the Fanconi anemia pathway*. Nat Genet, 2018. 50(8): p. 1132-39.