PEDIATRIC GERMLINE PREDISPOSITIONS TO MYELODISPLASTIC SYNDROME, EXPERT GUIDANCE FOR THE INITIAL EVALUATION AND MANAGEMENT OF SAMD9 AND SAMD9L VARIANTS, AND THE IMPORTANCE OF DATABASE DEVELOPMENT FOR THESE RARE SYNDROMES

by

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES .................................................................................. Page 6

ABSTRACT .................................................................................................................. Page 7

CHAPTERS

1. Introduction: Pediatric Germline Predispositions to Myelodysplastic Syndrome ......... Page 8
   1.1. Germline Mutations that Predispose to Myeloid Neoplasms without a Pre-existing Disorder or Organ Dysfunction
       1.1.1. CEBPA ................................................................. Page 8
       1.1.2. DDX41 ............................................................. Page 9
   1.2. Germline Mutations that Predispose to Myeloid Neoplasms with Pre-existing Platelet Disorders
       1.2.1. RUNX1 ............................................................. Page 11
       1.2.2. ANKRD26 ......................................................... Page 12
       1.2.3. ETV6 ................................................................. Page 13
   1.3. Germline Mutations that Predispose to Myeloid Neoplasms Associated with Other Organ Dysfunction
       1.3.1. GATA2 ............................................................... Page 14
   1.4. Germline Mutations that Predispose to Myeloid Neoplasms with Inherited Bone Marrow Failure Syndromes and Telomere Biology Disorders
       1.4.1. Fanconi Anemia ............................................... Page 16
       1.4.2. Diamond-Blackfan anemia ............................. Page 18
       1.4.3. Schwachman-Diamond syndrome.................. Page 19
       1.4.4. Severe Congenital Neutropenia ..................... Page 21
       1.4.5. Dyskeratosis congenita/Telomeropathies ......... Page 23

   2.1. Introduction ........................................................................................................ Page 25
   2.2. Methods ........................................................................................................... Page 26
   2.3. Recommendations:
       2.3.1. When to Suspect SAMD9/SAMD9L Syndromes ........................................ Page 27
           2.3.1.1. Hematologic Presentation ................................................................. Page 27
           2.3.1.2. Constitutional Abnormalities ............................................................ Page 28
           2.3.1.3. Positive Family History ................................................................. Page 28
       2.3.2. How to Confirm Diagnosis of SAMD9/SAMD9L....................................... Page 29
           2.3.2.1. Detection of SAMD9/SAMD9L variants ........................................ Page 29
       2.3.3. Recommended Hematologic Evaluation .................................................... Page 30
           2.3.3.1. Baseline Hematologic Evaluation .................................................... Page 30
           2.3.3.2. Baseline Bone Marrow Evaluation ................................................ Page 30
List of Tables and Figures:

**Figure 1:** Use of the Modified Delphi Approach Using Consensus-Based Guidance in Determining Recommendations for *SAMD9* and *SAMD9L*……………………………………Page 27

**Figure 2:** Clinical presentation of *SAMD9/SAMD9L* associated syndromes………………Page 28

**Table 1:** *SAMD9/SAMD9L* Germline Mutational Phenotype Definitions…………………………Page 33

**Figure 3:** Management of Patients with Pathogenic *SAMD9/SAMD9L* Germline Mutations…………………………………………………………………………………Page 36

**Table 2:** Data Variables for Bone Marrow Failure Patient Registry…………………………Page 41
Abstract

Bone marrow failure and hematologic malignancy is rare within the pediatric population. Germline mutations within this population were initially thought to be rare, but we are finding that these malignancies are more likely to have germline predispositions than initially thought.

*SAMD9/SAMD9L* activating (gain or change of function) germline heterozygous mutations are a severe rare blood disorder with malignant potential in need of greater understanding and acute guidelines for diagnosis and therapy. In November 2019, the St. Jude Children’s Research Hospital sponsored the first international symposium on *SAMD9/SAMD9L* mutations. Forty internationally recognized experts met with the goal to develop a consensus on the diagnosis, monitoring, and management of individuals diagnosed with *SAMD9/SAMD9L* associated syndromes and carriers within affected families. The group mission was to improve accuracy of diagnosis and the timely detection of complications. A manuscript was then created, using the modified Delphi approach, to reflect the group’s recommendations for the initial evaluation and management of patients suspected to have *SAMD9/SAMD9L* variants. This manuscript is the first step to improving care for patients with *SAMD9/SAMD9L* variants.

Future research involves a patient registry/natural history prospective study to establish the data required to support the treatment recommendations which are controversial. Database development is an essential part of ensuring these registries are effective at collecting the necessary data. Rare disease registries are essential to comprehensively increase knowledge about disease characteristics, the natural course of these diseases, and assess the long-term outcomes of the patients affected by them.
Chapter 1: Introduction

Pediatric Germline Predispositions to Myelodysplastic Syndrome

Bone marrow failure and hematologic malignancy is a rare event within the pediatric population. While most pediatric hematologic malignancy is of somatic origin, there is a percentage of patients, particularly those with myelodysplastic syndrome (MDS) and Acute Myeloid Leukemia (AML), who have predisposing genetic mutations. These germline mutations were initially thought to be very rare, but as more is uncovered, we are finding that these malignancies are more likely to have germline predispositions than initially thought [1].

Germline mutations are mutations that are inherited and present in all the patients’ cells as opposed to somatic mutations, which drive mutations within malignant cells but are acquired and only present within the specific cancer cells.

Germline mutations were initially always associated with syndromes, particularly Fanconi anemia and telomere biology disorders. It wasn’t until 2004, with the discovery of CEBPA mutations, that we started to discover non-syndromic germline mutations [2]. Since that time, multiple genes have been discovered that are now known to predispose patients to the development of some of these lesser-known hematologic malignancies.

Identification of these germline predisposition syndromes is imperative to the proper treatment of these patients. Patients with these underlying conditions have different responses to therapy and can have additional symptoms. Patients with these conditions often undergo stem cell transplant as part of their treatment process, and it is vital to ensure the patient’s donor does not have the same genetic mutation prior to transplant.

Presented below is an overview of the current known germline predispositions to MDS and AML, however, this field is growing exponentially and there are always new discoveries on the horizon.

SAMD9/SAMD9L, the focus of this thesis and manuscript, will be presented in detail in the body of this document.

1.1. Germline Mutations that Predispose to Myeloid Neoplasms without a Pre-existing Disorder or Organ Dysfunction

1.1.1. CEBPA

Overview:

The myeloid transcription factor CCAAT/enhancer binding protein-alpha (CEBPA) is an important mediator of granulocytic maturation in combination with other regulators of hematopoiesis including RUNX1, PU.1, and GATA factors [3]. Individuals may inherit or develop a de Novo germ line mutation of CEBPA in rare cases, predisposing them to development of acute myeloid leukemia. Germline mutations are usually found in the 5’ end of the gene, while a somatic mutation is generally identified at the 3’ end of the other allele acquired at the time of progression to AML [4]. Several studies have shown that patients with presumed sporadic CEBPA-mutated AML harbor germline mutations in 5-10% of cases [3].
Clinical Presentation:
Patients typically present with acute myeloid leukemia as children or young adults. AML is generally the primary presenting feature with no preceding blood count or phenotypic abnormalities [4].

Diagnosis:
Identification of biallelic CEBPA mutations within leukemic cells should prompt evaluation for germline inheritance of one of the alleles [4]. While cure rates without transplant are high, patients with this predisposition developed AML recurrence and it is important to identify family members with this germline mutation prior to transplant.

Laboratory Evaluation:
Patients are generally asymptomatic until the development of AML. It is important once a CEBPA mutation is identified to assess for germ line mutations.

Risk of Malignant Transformation:
While this disorder appears to have near-complete penetrance for development of AML, the prevalence overall is unknown due to limited numbers of asymptomatic/healthy individuals being screened [3,4]. Studies suggest that AML presents following the acquisition of a somatic CEBPA mutation in the opposite genetic terminus to that of the germline lesion. While studies are limited, data supports that founding genetic lesions may predetermined the acquisition of later mutations across individuals with a shared genetic background; meaning family members with the same underlying germline mutation show an almost identical molecular profile of tumors upon development of AML [3].

Clinical Management:
Overall, patients who developed AML with germline CEBPA mutation have a favorable prognosis. Treatment of the leukemia is generally per standard institutional guidelines with overall survival being greater than 65% [3]. However, patients with this mutation are noted to have multiple relapses with different mutations identified at recurrence, suggesting that relapses may in fact represent novel independent clones rather than true relapse [3,4]. Due to this, hematopoietic stem cell transplant, from matched unrelated donor or an appropriately screened relative should be a consideration following salvage therapy to decrease risk of recurrence [3].

1.1.2. DDX41
Overview:
Germline DDX41 mutations are a relatively recently described autosomal dominant familial MDS/AML syndrome [4]. It is currently the only example of a germline spliceosomal mutation in leukemia [5]. It is characterized by inherited mutations in the gene on chromosome 5 encoding the DEAD box RNA helicase DDX41. Development of leukemia is usually seen when the DDX41 mutation is biallelic, with one of these mutations being germline [4].
Clinical Presentation:

Compared to other MDS/AML patients with hereditary DDX41 mutations are generally older at the time of presentation, thus not being a major germline mutation affecting pediatric patients [5]. Average onset of disease is in the 6th decade of life. Usual presentation is with leukopenia (with or without other cytopenias or macrocytosis), hypocellular bone marrow with prominent erythroid dysplasia, and a normal karyotype [4]. Most common neoplasms reported are MDS (MDS with multilineage dysplasia, MDS with excessive blasts and MDS with isolated del(5q)) and AML. Infrequently other neoplasms have been reported including chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), and Hodgkin and non-Hodgkin lymphomas.

Diagnosis:

Identification of DDX41 mutations within myeloid cells should prompt evaluation for germline inheritance. Overall prognosis is poor and it is important to identify potential germline mutations to ensure appropriate screening for hematopoietic stem cell transplant donors.

Laboratory Evaluation:

Patients are generally asymptomatic until the development of cytopenias and MDS/AML. It is important once a DDX41 mutation is identified to assess for germline mutations.

Risk of Malignant Transformation:

These pathogenic germline variants are seen with relative frequency in healthy control cohorts, which suggests that this conveys a risk for myeloid neoplasia rather than a leukemogenic driver lesion [5]. Overall prevalence of this germline mutation is unknown however DDX41 mutations have been found about 1.5% of myeloid neoplasms and half of these patients have germline mutations [4].

Clinical Management:

Prognosis of patients with germline DDX41 mutations is generally poor. Patients frequently achieve remission with traditional chemotherapies, however they generally relapse. There has been evidence that patients may respond to lenalidomide from retrospective analysis [6]. Allogeneic hematopoietic stem cell transplantation from donors with DDX41 mutation enhances the risk of donor cell leukemia so donors, especially familial donors, need to be screened for DDX41 mutations.
1.2. Germline Mutations that Predispose to Myeloid Neoplasms with Pre-existing Platelet Disorders

1.2.1. RUNX1 Deficiency - Familial Platelet Disorder with Predisposition to Myeloid Leukemia

Overview:

RUNT-related transcription factor 1 (RUNX1) is a master regulator of hematopoiesis. It is one of the most frequent somatic chromosome translocations in pediatric leukemias (ALL, AML, CML) in addition to MDS. These somatic changes are generally associated with poor prognosis in AML and MDS. Germline RUNX1 mutations were first described in 1999 by Song et al [7]. Typically inherited in an autosomal dominant fashion with incomplete penetrance and variable expressivity [8]. RUNX1 is located at 21q22 and causative mutations are most often frameshift, nonsense, or deletion mutations that result in premature protein truncation [9].

Clinical Presentation:

Patients with a RUNX1 germline mutation may be asymptomatic throughout their lifetime. Other patients may be noted to have a familial platelet disorder with myeloid malignancies. Symptoms varied greatly between patients upon initial evaluation ranging from mild to severe thrombocytopenia, functional platelet defects leading to prolonged bleeding, and even development of MDS, AML or T-ALL [8,9].

Diagnosis:

Proper diagnosis of this germline predisposition is critical for patients with leukemia. Patients that initially present with persisting thrombocytopenia or have a family history of thrombocytopenia/easy bleeding, MDS, AML or T-ALL, should be evaluated for genetic predisposition.

Laboratory Evaluation:

Thrombocytopenia is usually mild to moderate. Platelet size is generally not affected. The thrombocytopenia is generally due to abnormal megakaryocyte maturation and polyploidization and impaired platelet formation. Dysmegakaryopoiesis is the most prominent abnormality in bone marrow smears and is evident generally before leukemic transformation [8].

Risk of Malignant Transformation:

In general, patients with a RUNX1 germline mutation have a 30%-40% risk of developing AML or MDS, or less frequently T-ALL. Onset of MDS/AML is on average 33 years with a wide range [9]. T-cell ALL generally develops a younger age [10].

Clinical Management:

Affected individuals generally do not require treatment for thrombocytopenia alone in the absence of clinical bleeding [9]. There are no clinical or laboratory markers currently available to predict when a patient will develop a malignancy, however recent data shows that clonal
hematopoiesis can be detected in >80% of asymptomatic patients by the age of 50 and may provide a future means of disease surveillance [11]. Clinical management of patients with RUNX1 mutations that developed MDS or AML is not different than those who do not carry a germline mutation. If a patient is to proceed towards hematopoietic stem cell transplantation however, potential familial donors would need to be screened for the mutation prior to being accepted as a donor. Those who also carry the mutation should not be used as donors as adverse outcomes including donor-derived leukemia and failure to engraft have been reported [10].

1.2.2. ANKRD26 (Thrombocytopenia 2)

Overview:

Germline ANKRD26 mutation is an autosomal dominant disorder characterized by moderate thrombocytopenia and increased risk of developing MDS/AML [4]. This disorder is associated with germline mutations in the 5’ untranslated region of the gene Ankyrin Repeat Domain 26 (ANKRD26) on chromosome 10p12 [9]. This disrupts the assembly of RUNX1 and FLI1 on the ANKRD26 promoter, resulting in increased gene transcription and signaling through the MPL pathway which leads to impaired proplatelet formation by megakaryocytes [4].

Clinical Presentation:

Platelet count upon presentation can be variable, however the thrombocytopenia is usually moderate (average platelet count was 48,000/mL) with a normal platelet volume and decreased alpha-granule content leading to a pale platelet appearance, decreased platelet surface membrane glycoprotein Ia (GPIa), elevated thrombopoietin levels, and variable platelet aggregation defects [12]. Bleeding tendencies and infected patients are usually mild [4].

Diagnosis:

Diagnosis should be considered in patients with family history of thrombocytopenia and myeloid malignancy. While the prevalence is not well described, and inherited thrombocytopenia registry identified ANKRD26 mutations in 11 percent of patients evaluated [13].

Laboratory Evaluation:

Bone marrow morphology can show dysmegakaryopoiesis with an increased number of hyposegmented micromegakaryocytes at baseline which can make it difficult to appropriately distinguished individuals with germline ANKRD26 mutations from dysmegakaryopoiesis secondary to the development of MDS [12]. A small number of patients have also been seen to have elevated hemoglobin concentration and leukocyte counts [12].

Risk of Malignant Transformation:

While patient numbers are low, the estimated prevalence of the development of myeloid neoplasm is approximately 30 times higher in families with this germline mutation than that of the general population [4]. Most reported cases are AML or MDS, however there is a smaller number of patients that have chronic myeloid leukemia, CMML or chronic lymphoid leukemia [12].
Clinical Management:

Management of patients with Thrombocytopenia 2 is similar to that of other familial platelet disorders with myeloid predisposition. It is generally recommended to perform routine surveillance with bone marrow biopsy and cytogenetics at diagnosis and evaluation and blood work at regular intervals. Upon development of leukemia, treatment is generally per institutional standard, however as with other inherited predispositions to leukemia genetic testing should be performed in all family members prior to choosing a donor for stem cell transplant.

1.2.3. ETV6 (Thrombocytopenia 5)

Overview:

Germline ETV6 mutation (Thrombocytopenia 5) is an inherited autosomal dominant MDS/AML predisposition syndrome, similar to that of RUNX1 and ANKRD26 mutations. ETV6 was originally discovered as a leukemia-associated chromosomal translocation that has been identified as a fusion partner in more than 30 chromosomal translocation oncogenes [14]. More recently, ETV6 has been uncovered as a germline mutation in families with propensity to develop hematologic malignancy of a diverse nature including MDS, AML, CMML, B lymphoblastic leukemia, and plasma cell myeloma. Non hematologic neoplasms have also been noted including colorectal adenocarcinoma within these families [4]. Mutation is caused primarily by missense mutations in the gene ETV6, located on chromosome 12p. These mutations have a dominant negative function, disrupting the nuclear localization of the ETV6 protein and resulting in reduced expression of platelet associated genes [9].

Clinical Presentation:

Patients present with a variable degree of thrombocytopenia, with platelet counts ranging from <50 to 150 x 10^9/L, with generally normal sized platelets [9,14]. Severe thrombocytopenia (<20 x 10^9/L) was rare in the absence of MDS [14]. Patients have mild to moderate bleeding tendencies, generally including epistaxis, gum bleeding, easy bruising, and menorrhagia [14]. Occasionally symptoms can present in infancy [4]. While a variety of physical anomalies have been reported in a few patients with germline ETV6 mutations, no characteristic medical findings other than increased bleeding propensity were reported. Since there are still an overall small number of reported cases it is unclear whether these additional features are associated with the ETV6 germline mutation or not [14].

Diagnosis:

As with all prior familial platelet disorders with predisposition to malignant transformation proper diagnosis is very important so genetic testing should be considered in all patients with thrombocytopenia and malignancy.

Laboratory Evaluation:

There are a limited number of bone marrow biopsies on patients without leukemia, however these have demonstrated small hyosegmented megakaryocytes and mild dyserythropoiesis.
Risk of Malignant Transformation:

While the true prevalence of this germline mutation is unknown and numbers of identified families is low, it is not possible to accurately define the risk of malignant transformation. However, there are several recent studies that give us an indication of this risk. Targeted sequencing of ETV6 in a recent study of 4405 childhood ALL cases identified 31 germline variants potentially related to leukemia in 35 cases (0.79%) [15]. Four other studies with a combined patient number of 41 subjects found that 16 patients (39%) developed hematologic malignancies, with 12 of those patients (29%) developing ALL [16]. All but one of these patients were children. A subsequent study found that four of 20 consecutive patients with ETV6-RT (20%) developed ALL during childhood – confirming that early leukemic transformation is a major risk in these patients [16].

Clinical Management:

As with the other hereditary familial platelet disorders, it is recommended to perform routine surveillance with bone marrow biopsy and cytogenetics at diagnosis and evaluation and blood work at regular intervals. Upon development of leukemia, treatment is generally per institutional standard, however as with other inherited predispositions to leukemia, genetic testing should be performed in all family members prior to choosing a donor for stem cell transplant, to avoid using family members who also carry the mutation as the donor [10].

1.3. Germline Mutations that Predispose to Myeloid Neoplasms Associated with Other Organ Dysfunction

1.3.1. GATA2 Deficiency

Overview:

GATA2 is a zinc-finger transcription factor nuclear regulatory protein that is involved with regulating haematopoiesis, autoimmunity, inflammatory and development processes [4]. It is highly expressed in immature hematopoietic cells and declines with blood cell maturation. It is crucial for the proliferation and maintenance of hematopoietic stem cells and the description of its activity can contribute to leukemogenesis [17]. It is also important in the production of megakaryocytes, mast cells, natural killer (NK) cells and monocytes [18]. Germline pathogenic variants include deletions, missense, nonsense, frameshift, and splice site changes and alterations of intronic regulatory elements. All mutations mechanistically (hemizygosity, transcription of the cis allele, nonsense mediated decay, premature stop codons, or splice site alterations) lead to haploinsufficiency [18]. Most pathogenic variants cluster in its 2 zinc fingers, leading to nonfunctional protein unable to bind DNA or other transcription factor partners.

GATA2 deficiency was first described in 2011 as a major myelodysplastic syndrome predisposition syndrome, accounting for 15% of advanced and 7% of primary MDS cases [19]. Initially, it had been identified as four separate syndromes described by their constellation of symptoms including 1) monocytopenia and mycobacterium avium complex (MonoMAC), 2) dendritic cell, monocytes, B and NK lymphoid deficiency (DCML), 3) Emberger syndrome, and
4) familial MDS/AML. However, now due to considerable phenotypic overlap in patients they are now recognized as a single genetic disorder referred to as GATA2 deficiency [4,17,18,20].

Clinical Presentation:

Patients tested and diagnosed at birth, due to knowledge of an affected family member, are immunologically and hematologically normal [18]. Symptomatic clinical presentation is variable, with multiple phenotypes ranging from mild cytopenias to severe immunodeficiency (warts, atypical mycobacterial infections, herpes virus infection, fungal disease) and myeloid neoplasia [9]. Some patients also have syndromic features, such as congenital deafness and lymphedema (Emberger syndrome) or pulmonary disease and vascular problems. Patients have also been described as having hypertelorism, epicantthic folds, long tapering fingers, neck webbing, behavioral disorder/ADHD, hypothyroidism, urogenital malformations, ptosis or hydrops fetalis [17]. Anemia and thrombocytopenia are uncommon in early presentation [18]. Profound monocytopenia is one of the most consistent features, however this is usually later in disease development [18]. Patients with MDS and a history of prior immune deficiency or a lack of B cell and B-cell progenitors is a strong indicator of a GATA2 mutation [17,18].

Diagnosis:

GATA2 haploinsufficiency is diagnosed by full gene sequencing and large rearrangement testing [4].

Laboratory Evaluation:

Lymphocyte subset evaluation may identify B and NK cell cytopenias, however pediatric MDS with germline GATA2 mutation can present without recognized immunodeficiency as the immune system is competent for sufficiently long to establish a level of immunologic memory [20]. Bone marrow evaluation is typically hypocellular with characteristic features, including a spectrum of atypical megakaryocytes. Immunohistochemistry for CD61 on core biopsy may help identify megakaryocytic atypia. Review of aspirate smears is necessary to assess for progression to MDS [18]. MDS with GATA2 deficiency is often accompanied by cytogenetic changes, including monosomy 7 (68% of patients), trisomy 8 (9% of patients), and trisomy 1q (7% of patients) [19]. It critical for all pediatric patients with MDS and AML with a somatic GATA2 mutation to be screened for germline mutations prior to stem cell transplant.

Risk of Malignant Transformation:

Approximately 75% of patients will develop a myeloid neoplasia (myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia, acute myeloid leukemia) [18]. The average age of onset of myeloid neoplasia varies depending on the study but ranges from 12 to 35 years old, with the median age of onset being 19.7 years [4,9].

Clinical Management:

While there are no standard guidelines for patient care and monitoring, expert opinion is to monitor known patients with twice yearly CBCs and yearly lymphocyte subset panels, bone marrow evaluation, pulmonary function testing, skin examination, and gynecologic examination
In general, immunosuppressive therapy seems to increase risk and delays definitive treatment. HSCT is currently the only curative option for patients with GATA2 deficiency [18]. While there is no evidence to support HSCT in GATA2 mutation carriers who are phenotypically silent, HSCT should be performed before patients develop MDS-ED with karyotypic abnormalities, severe immunodeficiency with systemic infections, or severe lung disease. Ideal time for HSCT is considered during the phase with marrow hypocellularity and before manifestation of severe complications or development of monosomy 7 and/or blast increase [17].

1.4. Germline Mutations that Predispose to Myeloid Neoplasms with Inherited Bone Marrow Failure Syndromes and Telomere Biology Disorders

1.4.1. Fanconi anemia

Overview:

Fanconi anemia (FA) is a rare, inherited predisposition to bone marrow failure [9]. It is estimated at 1:360,000 births. FA is caused by homozygous or compound heterozygous mutations in the FA complement groups [9]. There are at least 20 genes that are known to cause FA, with the most common being FANCA with 65% of cases, followed by FANCC accounting for 14% of cases [9,21]. FA genes are transmitted via autosomal recessive inheritance, except for FANCB which is transmitted via an X-linked pattern [21]. Proteins encoded by FA genes have important roles in many cellular functions including DNA repair, detoxification of reactive oxygen species and aldehydes, energy metabolism, and both proinflammatory and myelosuppressive cytokine homeostasis [21].

Clinical Presentation:

Fanconi anemia is a heterogenous disorder with a large range of clinical manifestations including congenital anomalies (low birth weight, short stature, radial anomalies, congenital heart disease, microphthalmia, ear anomalies, characteristic facies, cataracts, deafness, kidney and urinary tract malformations, hypogonadism, numerous café-au-lait spots), bone marrow failure, and other solids tumors [4,9,21]. Congenital anomalies consistent with the VACTRL (vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula with esophageal atresia, renal and limb abnormalities) or VACTRL-H (with hydrocephalus) constellation are frequently associated with FA due to variants in FANCD1/BRCA2 or FANCI [21]. Patients may also develop metabolic defects, endocrinopathies (pituitary/thyroid dysfunction or glucose intolerance), cataracts and hearing impairment [21]. Pancytopenia is often initially associated with increased fetal hemoglobin and macrocytosis. Marrow failure may begin with thrombocytopenia and progress to decline of white and red blood cell counts leading to aplastic anemia [21].

Up to 40% of individuals with FA lack physical abnormalities typically associated with the disease, and due to this these patients are more likely to develop MDS/AML as the presenting symptom of the underlying inherited syndrome [9]. Excessive toxicity after chemotherapy or radiation therapy for MDS/AML may by a primary presenting feature of FA [21].
Diagnosis:

Fanconi anemia is characterized by increased chromosomal fragility and breakage when treated with cross-linking agents (diepoxybutane or mitomycin C), therefore diagnosis is confirmed by chromosomal fragility testing [9]. Excessive chromosomal breaks and classic tri- and quadri-radial figures are seen in positive tests [21]. In some cases (somatic mosaicism or anomalous presentation) peripheral blood lymphocyte assays will be negative and chromosomal fragility tests will need to be performed on skin fibroblasts at specialized laboratories. Next-generation sequencing, whole-exome/genome sequencing are now also possible techniques for diagnosis [21].

Risk of Malignant Transformation:

Progressive bone marrow with pancytopenia usually occurs in the first decade, and by age 50, an estimated 90% of patients with FA have bone marrow failure [9]. FA carries a 600 to 800-fold increased risk for development of AML and 6,000-fold increase for MDS than that of the general population [4], with the incidence of hematologic malignancies to be 10-30% overall with certain sub-groups having higher cumulative incidence (ie 80% by age 10 in the FANCD1/BRCA2 sub-group). FA also carries an increased risk of solid tumors, particularly squamous cell carcinomas of the head and neck [9].

Laboratory Evaluation:

Patients with FA require a surveillance plan for monitoring hematopoietic function and for potential clonal evolution. A baseline bone marrow needs to be performed at diagnosis, with evaluation for dysplastic changes, presence of blast cells, cytogenetic abnormalities, and leukemic mutations [21].

Clinical Management:

FA patients generally require close monitoring for development of hematologic abnormalities. Yearly evaluation in a hematologically asymptomatic patient is recommended. If patients develop a chromosomal abnormality this becomes more frequent, and overall course of therapy depends on risk group of the chromosomal abnormality [22].

Stem cell transplantation (SCT) is the only definitive treatment for bone marrow failure with high-risk chromosomal abnormalities, MDS and AML in patients with FA. SCT currently offers a 30-40% long term overall survival rate [22]. Timing, source of hematopoietic stem cells and condition regimen for SCT need to be very carefully considered [21]. Ideal time for SCT in FA if patient has a matched donor is when the patient has clearly progressive cytopenia with need for transfusion support prior to clonal evolution development [22]. SCT becomes necessary with overt AML/MDS with excessive blasts, significant dysplasia and/or poor-prognosis cytogenetic abnormalities (+3q, -7q, RUNX1-abn, and/or complex) [21].

Patients with FA require reduced-intensity conditioning for stem cell transplantation [9] due to their sensitivity to DNA-damaging agents. Chemotherapy is regularly associated with significant toxicity and possible prolonged period of aplasia, which in some cases could also lead to SCT.
being contraindicated, however necessitates a donor for SCT being selected prior to offering chemotherapy in these patients [22].

1.4.2. Diamond-Blackfan anemia

Overview:

Diamond-Blackfan anemia (DBA) is an inherited bone marrow failure syndrome characterized by pure red cell aplasia and congenital anomalies, arising from pathogenic mutations in ribosomal proteins [22]. Pathogenic variants have been found in over 20 genes [23]. 25% of all cases of DBA arise from mutations in RP S19, encoded by RPS19 which is a component of the small ribosomal subunit. This was the first gene discovered and provided a link to ribosome biology which led to the discovery of other heterozygous mutations or partial deletions in components of the 60S large ribosomal subunit (RPL35A, RPL5, and RPL11, RPL26, RPL15, RPL31, and RPL27) and 40S small subunit (RPS17, RPS24, RPS10, RPS7, and RPS26, and RPS29) [23]. DBA is most often inherited in an autosomal dominant manner, however GATA1-related and TSR2-related DBA have been shown to be inherited in an X-linked manner.

Approximately 40-45% of individuals with DBA have inherited the pathogenic variant from a parent, with the other 55-60% developing a de novo pathogenic variant [23].

Clinical Presentation:

DBA in its classic form is characterized by a profound normochromic and usually macrocytic anemia with normal leukocytes and platelets. The phenotypic spectrum ranges from a mild form (mild anemia/subtle erythroid abnormalities vs physical malformations without anemia) to a severe form of fetal anemia resulting in nonimmune hydrops fetalis. Hematologic complications are the most common symptom and occur in 90% of affected individuals within their first year of life. Congenital malformations are identified in 50% of individuals and include head and face anomalies (Microcephaly, hypertelorism, epicanthus, ptosis, microtia, low-set ears, broad, depressed nasal bridge, cleft lip/palate, high arched palate, micrognathia, low anterior hairline, glaucoma/cataracts, strabismus), neck webbing/short neck, Klippel-Feil anomaly, Sprengel deformity, limb abnormalities (absent radial artery; flat thenar eminence; triphalangeal, duplex, bifid, hypoplastic, or absent thumb), GU defects (absent kidney, horseshoe kidney, hypospadias), and cardiac anomalies (atrial and ventricular septal defects, ASD, coarctation of the aorta). 30% of individuals are affected by growth retardation [24]. DBA is associated with an increased risk for acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), and solid tumors including osteogenic sarcoma [23].

Diagnosis:

Diagnosis of DBA should be considered in patients with anemia and failure to thrive with congenital malformations. DBA is established when all four of the diagnostic criteria are met [23]: 1) age younger than one year; 2) macrocytic anemia with no other significant cytopenias; 3) reticulocytopenia; and 4) normal marrow cellularity with a paucity of erythroid precursors. Molecular testing for pathogenic variants can also establish the diagnosis of DBA. Generally,
sequence analysis of RPS19 is performed first, followed by sequence analysis of the remaining genes if this is negative [23].

Laboratory Evaluation:
Upon suspicion of DBA, an individual should have a CBC with reticulocyte count, erythrocyte adenosine deaminase activity, fetal hemoglobin and bone marrow aspiration and biopsy performed. Upon diagnosis patients should be followed with frequent CBC’s (several times a year) with bone marrow evaluation with development of cytopenias to determine presence of acquired abnormalities associated with malignancy [25].

Risk of Malignant Transformation:
While the numbers of malignancies from DBA is lower than that of FA or DC it still carries a significantly elevated risk of malignancy over the general population [26]. Quantification of the absolute risks in DBA is limited due to its rarity [21], however the Diamond Blackfan Anemia Registry (DBAR), the largest established DBA patient cohort established in 1991, has published recently updated observed-to-expected (O/E) ratios for all combined cancers to be 4.75, with significant O/E ratios of 352 for MDS and 29 for AML. They also noted significant increased risk for both colon carcinoma and osteosarcoma [21,25].

Clinical Management:
Treatment for patients who have a pure red cell aplasia without malignant transformation includes packed red blood cell (pRBC) transfusions and corticosteroids. Oral steroids have been shown to induce remission of anemia in up to 80% of patients [21]. Patients who are resistant to corticosteroid therapy require chronic pRBC transfusions [23]. This comes with the risk of iron overload, so is usually accompanied by iron chelation. Spontaneous remission of anemia has been noted in up to 25% of patients. Hematopoietic stem cell transplantation (HSCT) is the only curative therapy for DBA and is usually reserved for those unresponsive to other therapies or who develop MDS. Overall survival was best in those patients who were under 10 years old and had a matched-sibling donor [23]. All potential familial donors need to be screened genetically for DBA, since it is possible that a relative could have a pathogenic variant without manifesting findings of DBA. Relatives with a pathogenic variant should not be used as donors [23].

1.4.3. Schwachman-Diamond syndrome
Overview:
Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder, characterized by pancreatic insufficiency and bone marrow failure which is frequently seen with neurodevelopmental and skeletal abnormalities [27]. 90-95% of all cases are caused by a pathogenic biallelic or homozygous mutation that occurs in the SBDS (Shwachman Bodian Diamond syndrome) protein. How SBDS connects to hematopoiesis is still an area of investigation, however there is evidence this is linked to cell proliferation, mitosis, and maintaining the stromal microenvironment [28], and it is known that SBDS functions in
ribosome biogenesis through joining the 60S and 40S ribosomal subunits to allow for protein synthesis [21].

Clinical Presentation:

The main clinical features of SDS are bone marrow failure, pancreatic exocrine dysfunction, and skeletal abnormalities (short stature, delayed appearance of secondary ossification centers, metaphyseal widening and dysostosis, osteopenia, Wormian bones of the skull, and thoracic bony abnormalities) [21,26]. Failure to thrive, secondary to pancreatic insufficiency, during infancy may be the presenting symptom [21]. Neutropenia may also be significant early on, and is the most common hematological abnormality, occurring in nearly all patients. The degree and frequency of neutropenia however can vary, ranging from mild to severe and intermittent (66%) to persistent (33%) [26,29]. Neutropenia is frequently followed by bilineage or trilineage cytopenias [21]. Anemia with low reticulocyte count is present in up to 80% of patients [26], appearing normochromic and normocytic generally. Fetal hemoglobin will also be elevated in approximately 80% of patients. Thrombocytopenia is more variable.

Diagnosis:

Initial suspicion of diagnosis occurs with patients with pancreatic insufficiency (low serum trypsinogen in children under 3 years or low serum isoamylase in children over 3 years, and/or elevated fecal fat excretion in over 72 hours) in combination with neutropenia and bony abnormalities. These abnormalities are only seen however in 65% of patients [29]. Genetic testing for autosomal recessive inheritance of pathogenic variants in SBDS however is confirmation in 90-95% of patients with SDS [21]. It is still unclear if those remaining 10% of patients with clinical implications of SDS, but with negative genetics represent a yet unidentified gene for SDS, or if they represent a separate disorder entirely [29]. It is also unclear what the significance of a positive SBDS mutation is in patients who are asymptomatic or do not carry a positive family history [30].

Laboratory Evaluation:

Peripheral blood counts should be monitored every 3-4 months to identify cytopenias early [31]. Bone marrow biopsy should be obtained on patients with suspected SDS and repeated yearly to assess for marrow cellularity, MDS, acute leukemia, or other clonal disease [30].

Risk of Malignant Transformation:

There are several studies that estimate the overall risk of developing malignancy with SDS. A report (Donadieu et al) described 55 patients with SDS from the French Severe Chronic Neutropenia Registry [32]. 7 patients within this cohort developed MDS or AML, estimating the risk of developing MDS or AML at 19% by 20 years of age and 36% by 30 years or age [31].

Marrow cytogenetic clonal abnormalities, particularly involving chromosome 7 (monosomy 7, der(7), i(7q) and del(20q)), have commonly been reported with SDS. The significance of these abnormalities on development of MDS and AML is still being investigated. There have been no
reports of progression to AML among SDS patients with i(7q) abnormalities, however 42% of patients with other chromosome 7 abnormalities progressed to advanced MDS or AML [33].

Clinical Management:

Treatment for SDS is usually medical, giving replacement pancreatic enzymes and fat-soluble vitamins to correct for pancreatic insufficiency, and granulocyte colony-stimulating factor (G-CSF) to improve neutrophil counts. There is no evidence to date that treatment with G-CSF increases risk of malignancy in patients with SDS [29]. Antimicrobials are usually given prophylactically to prevent infections during periods of neutropenia [21]. There are very limited patients with SDS who progress to need HSCT however this is the only definitive therapy for marrow failure, MDS, or AML [30]. Poor outcomes have been reported following HSCT due to graft failure/rejection, toxicity, and relapsed disease. Cardiac and organ toxicities have been reported, thought to be worsened by the intensive preparative regimens on top of underlying organ dysfunction [30]. Currently indication for HSCT with SDS includes severe persistent or symptomatic cytopenia, MDS with excess blasts, and overt leukemia. Generally reduced intensity conditioning regimens should be considered for these patients [30].

1.4.4. Severe Congenital Neutropenia

Overview:

Severe congenital neutropenias (SCN) are a heterogenous group characterized by impaired maturation of neutrophil granulocytes [34], leading to severe neutropenia (absolute neutrophil count of <500/µL). Because of this defect patients are susceptible to recurrent, life-threatening infections starting early in life, with usual presentation in the first year of life [33,34]. There are multiple pathogenic variants, in several genes, that are transmitted primarily via autosomal dominant or autosomal recessive patterns [33]. De novo mutations may also occur [21]. Mutations within the ELANE gene, which encodes for neutrophil elastase, are found in over 50% of patients with SCN in both European and North American Registries [21]. There are clear demographic mutational differences within SCN populations. HAX1 mutations account for 11% of European cases, however there are no reported HAX1 mutations in the United States [33]. In Israel there is a unique pattern of mutations with a 25% prevalence of G6PC3 mutations found [33].

Less common genes that have been identified to cause SCN include pathogenic variants in GFI1, WASP, G6PC3, VPS45, JAG1, and TCRG1 [33].

Clinical Presentation:

Patients with SCN usually present early in childhood with severe neutropenia and recurrent infections. Acute and severe umbilical infections may be seen in the first days of life [33]. Febrile respiratory infections within the first weeks of life or skin infections during the first few months are also indictors of SCN. Severe gingivitis and periodontitis can also develop during the patients first two years. The majority of patients are diagnosed within the first 6 months of life [24], however there are often delays between the first symptoms and confirmation of diagnosis due to lack of obtaining a CBC with infections or neutropenia being dismissed as an autoimmune
disorder [33]. Most patients however are more severely ill than would be expected, and recurrent and painful mouth sores are an important clue to prompt blood cell counts. While neutropenia is the most significant manifestation other features of SCN can include organ failure, diarrhea, malabsorption, and failure to thrive [33].

Diagnosis:

When the diagnosis of SCN is suspected patients should undergo complete blood count evaluation at least 3 times a week for 2-4 weeks to rule out cyclic neutropenia [34]. Bone marrow evaluation should be completed as an early diagnostic step. Typical bone marrow findings in patients with SCN are elevated numbers of promyelocytes and myelocytes and paucity of metamyelocytes, band cells and mature neutrophils [33]. Molecular testing for genetic mutations can be used to confirm and classify the diagnosis [34]. It is reasonable to sequence ELANE first, then branch to the other commonly associated genes. If negative, branching to a panel of neutropenia-associated genes or whole exome sequencing would be the next step. Approximately 22% of patients, based on the European database, do not have a currently known SCN associated genetic mutation, so negative testing does not exclude SCN as a diagnosis.

Risk of Malignant Transformation:

Patients with SCN are at increased risk of developing MDS/AML. Cumulative incidence of MDS/AML is estimated to be 11% by 20 years of age and 22% after 15 years of G-CSF. While G-CSF significantly reduces the number of life-threatening infections and improves survival in patients with SCN, it is likely that the G-CSF affects clonal evolution, increasing the risk of evaluation to MDS/AML [21]. The effects of this do seem to be dose dependent with doses greater than 8 µg/kg/day being associated with highest risk [35]. Development of a somatic CSF3R mutation, which results in truncation of amino acids in the cytoplasmic domain of G-CSFR which leads to blocked G-CSF-induced neutrophil differentiation and maturation. This acquired clonal mutation was found in >80% of patients with SCN who developed leukemia vs 30-35% of patients with SCN who did not, supporting the association that acquisition of CSF3R mutations and leukemia [33].

Clinical Management:

G-CSF is usually first line therapy in all patients with SCN. The goal is to achieve and maintain ANC >1000 x 10^9/L. Blood counts need to be assessed bi-weekly until an appropriate dose is reached and then monitored several times a year to maintain appropriate treatment [33].

With the efficacy of G-CSF therapy, HSCT is no longer essential for saving lives of patients with SCN. HSCT is still indicated however in patients who do not respond to G-CSF (dose of G-CSF is >50 µg/kg/day with ANC <0.5 x 10^9/L) or those who develop AML or MDS. Prior to 2000 all patients who were treated for MDS/AML with SCN and did not receive HSCT died, and those who were treated with chemotherapy prior to HSCT had poor outcomes with transplant-related infectious complications and graft-vs-host disease [36]. Since then reduced intensity regimens have improved outcomes substantially, by going directly to HSCT as soon as MDS/AML develops [37].
1.4.5. Dyskeratosis congenita/Telomeropathies

Overview:
Dyskeratosis congenita (DC) is the prototypic telomere biology disorder. It represents a spectrum of disorders caused by pathogenic germline variants in telomere biology genes [22]. DC is inherited in X-lined recessive, autosomal dominant, and autosomal recessive forms [38]. The first gene that made the link to the role of telomere biology in DC pathogenesis were X-linked pathogenic variants in dyskerin, encoded by *DKC1* [38]. These cause very short telomeres in patients with DC [21]. Incomplete replication of the 3’ ends of DNA leads to shortening of these patients’ telomeres, the nucleotide repeats and protein complex at chromosome ends which are essential for chromosome stability. Autosomal dominant forms of DC have been found to be due to mutations in *TERT* and *TERC* [21]. These result in short telomeres due to haploinsufficiency of telomerase [38]. DC is also caused by heterozygous pathogenic variants in components of the shelterin telomere protection complex (*TINF2, ACD, POT1*), and in the DNA helicase and telomere biology protein, *RTEL1* [21]. Autosomal recessive inheritance, which comprises the smallest group of patients [38], is due to homozygous or biallelic pathogenic variants in other components of the telomerase holoenzyme complex, *NHP2* and *NOP10*, the telomerase-associated cajal body-associated protein TCAB1, a telomere capping protein, poly(A)-specific ribonuclease (PARN) CTC1, in addition to *TERT* and *RTEL1* [21].

Clinical Presentation:
DC was originally characterized by a diagnostic triad of nail dystrophy, abnormal reticular skin pigmentation, and oral leukoplakia, however, these features are not always present and may or may not develop over time [38]. There is now knowledge of a wider spectrum of disease presentation and these symptoms develop at different ages and rates even within families [21]. Patients are at high risk of bone marrow failure, hematopoietic (MDS/AML) and solid (squamous cell carcinomas of the head, neck and anogenital tract) malignancies, pulmonary fibrosis, non-alcoholic/non-infections liver disease, avascular necrosis of the hips and shoulders, stenosis of the lacrimal ducts, esophagus, and urethra, and immunodeficiency [9,21]. Patients may also have dental caries, esophageal strictures, or premature hair loss or greying [39]. Bone marrow failure is often the initial presenting symptom and is the leading cause of death in affected patients. Patients with *TERT* and *TERC* mutations are associated with anticipation, showing progressively shorter telomeres with each subsequent generation [39].

Diagnosis:
DC can be difficult to diagnose due to the variety of phenotypic presentations, and large age spectrum at which symptoms begin [21]. Clinical diagnosis is based on presence of at least two of four major features of the disease (nail dystrophy, abnormal reticular skin pigmentation, oral leukoplakia, and bone marrow failure) in addition to two multisystem features of the disease (epiphora, learning difficulties, pulmonary disease, short stature, extensive dental carries, esophageal stricture, premature hair greying, hyperhidrosis, or malignancy) [39]. Confirmatory diagnostic testing is performed by testing telomere length. DC is now characterized by very short telomeres (<1% of age-matched normal controls) determined by multicolor flow cytometry.
fluorescence in situ hybridization (flow-FISH) on white blood cell subsets [9] and telomeres <1% in three out of five lymphocyte subsets is >95% sensitive and specific for diagnosing DC [21]. Pathogenic germline mutations can be detected in only 50-60% of patients with a clinical diagnosis of DC, so this is not used for diagnostic purposes [27,39].

Risk of Malignant Transformation:

Due to limited patient numbers the absolute risk of malignancy in DC is not clear [21]. Age of onset of malignancy varies by report. A 2009 study reported the median age of onset in 52 patients with DC to be 29 years for all cancers, while the National Cancer Institute (NCI) reporting the median age at onset of first malignancy is 37 years (25-44), with the median age of onset of MDS being 35 years (range 19-61) [40]. An update of malignancies in 197 DC patients in the NCI cohort found a cumulative incidence of cancer of only 2% by age 50 years for leukemia, 11% by 50 years for solid cancers [41].

Laboratory Evaluation:

Bone marrow failure in these patients can progress at varying rates so management is patient specific [21]. A baseline bone marrow evaluation should be performed at diagnosis [21]. If patients progress to having significant cytopenias, evaluation should occur at least yearly including peripheral and bone marrow evaluation [21]. Patients with TERT and TERC mutations will need to be screened more closely than other DC patients due to anticipation and younger generations having more severe disease at earlier ages [39].

Clinical Management:

SCT is the only definitive cure for patients with DC who develop bone marrow failure/malignancy. There is a higher rate of post-transplant complications (graft failure, increased risk of graft-vs-host disease, sepsis, pulmonary fibrosis, hepatic cirrhosis, and veno-occlusive disease) partially due to underlying pulmonary and liver disease [9]. Patients with DC are at a much greater risk with conventional myeloablative conditioning regimens, so screening patients for DC prior to transplant is extremely important [39] and these patients should receive a reduced-intensity conditioning regimen.

Oxymetholone is also a medication that may improve hematopoietic function in some patients through upregulation of telomerase [39].
Chapter 2: Body

Expert Guidance for the Initial Evaluation and Management of SAMD9 and SAMD9L Variants

2.1. Introduction

SAMD9 and SAMD9L (SAMD9/SAMD9L) are single-exon paralogue genes, located on the inverse strand of chromosome 7 (chr7) at the 7q21.1 cytoband [42]. The expression levels of both genes were tightly regulated during embryonic development, switching to ubiquitous levels in adult tissues [43, 44]. However, the distinct function of both genes remains speculative, with a general role as negative regulators of cellular proliferation. Heterozygous SAMD9/SAMD9L patient variants render to be gain-of-function with enhanced induction of growth arrest [1, 45-49] and apoptosis [50, 51] in vitro. Clinically these variants lead to autosomal dominant syndromes with incomplete penetrance and overlapping features, affecting the neurologic, endocrine, immunologic, and hematologic systems [1, 45-47, 49, 51-68]. The initial presenting symptoms of patients with SAMD9/SAMD9L variants are diverse; can range from a constellation of symptoms named MIRAGE syndrome (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) [45, 46] in cases with SAMD9 variants to progressive neurological anomalies with recurrent evidence of cerebellar ataxia in SAMD9L-associated ataxia-pancytopenia (ATXPC) [47, 52]. Other phenotypes that were recently added to the disease spectrum of SAMD9/SAMD9L were panniculitis, resembling CANDLE [65] (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperatures) or B-cell aplasia [50], and missense SAMD9mut in steroid-resistant nephrotic syndrome [68]. The common denominator of the different SAMD9/SAMD9L clinical subtypes is the hematological aberrations, which can also occur as the sole manifestation including non-syndromic familial myelodysplastic syndrome (MDS) and aplastic anemia (AA) [1, 48, 58, 66]. Cohort studies have further demonstrated germline SAMD9/SAMD9L alterations to account for a large percentage of pediatric MDS. Based on three independent studies, the prevalence can range from 8% (42/548) in a multi-institutional consecutive cohort of European Working Group of Childhood MDS (EWOG-MDS) [69] to 18% (16/86) in French national cohort of suspected bone marrow failure (BMF) syndromes [66] and 17% (18/46) in a single institution report [1].

Taken together, 66 distinct germline SAMD9/SAMD9L variants have been reported to date in 132 patients [1, 45-47, 49, 51-68]. The leukemic progression observed in SAMD9/SAMD9L mutated patients is not a direct implication of the underlying germline variant but rather an outcome of “compensatory aneuploidy” involving non-random loss of partial or complete chromosome7 (-7) containing the mutated SAMD9/SAMD9L allele. The -7 subsequently function as a (pre) malignant event with a predisposition to acquire additional somatic mutations in known leukemia genes like SETBP1, ASXL1, ETV6, and RAS pathway genes [1, 49, 58, 70].

Cumulatively, it is evident that there is a need for in-depth understanding and guidelines to both diagnose and treat patients with these genetic conditions. Hence, accurate and timely diagnosis of SAMD9/SAMD9L is vital to determine the natural history of these disorders and to develop evidence-based clinical standards-of-care.
### 2.2. Methods

A Steering Committee identified activating (gain or change of function) germline heterozygous \textit{SAMD9/SAMD9L} mutations deemed a severe rare blood disorder with malignant potential in need of greater understanding and acute guidelines for diagnosis and therapy. In November 2019, the St. Jude Children’s Research Hospital sponsored the first international symposium on \textit{SAMD9} (sterile \(\alpha\)-motif domain-containing protein 9) and \textit{SAMD9L} (\textit{SAMD9-like}) mutations. Forty internationally recognized experts met to achieve a consensus on the diagnosis, monitoring and management of individuals diagnosed with \textit{SAMD9/SAMD9L} associated syndromes and carriers within affected families. The group’s mission was to improve accuracy of diagnosis and the timely detection of complications. This manuscript reflects the group’s recommendations for the initial evaluation and treatment of patients suspected to have \textit{SAMD9/SAMD9L} variants.

In 2010 the American Society of Clinical Oncology (ASCO) approved development of guideline recommendations using consensus methodology [71]. The modified Delphi approach was selected for determining recommendations when traditional evidence-based recommendations cannot be developed due to a weak evidence base identified by systematic review. The modified Delphi approach consists of six steps: 1) generating draft recommendations, 2) panel meeting of experts, 3) consensus round one: ratings, 4) consensus round one: review results, 5) consensus round two: ratings, and 6) evaluation of consensus.

We utilized the modified Delphi approach to develop expert opinion consensus statements regarding the diagnosis and treatment of patients with \textit{SAMD9/SAMD9L} (Figure 1). Subspecialty fields identified to provide multidisciplinary consensus included hematology, oncology, bone marrow transplant, endocrinology, neurology, and genetics. On November 1, 2019 an international meeting was hosted by St. Jude Children’s Research Hospital [72]. Forty specialists from around the world attended including both basic scientists and clinicians who have been studying \textit{SAMD9} and \textit{SAMD9L} variants. The first day of the meeting consisted of review of the literature, review of current disease models, and review of current international experience with \textit{SAMD9/SAMD9L} families (step 1). On day two, moderators summarized diagnostic and treatment statements drafted by the steering committee, followed by discussion and debate (step 2). Following the meeting, statements were modified and distributed back to the expert panel via an anonymous REDCap survey where group members documented their agreement or disagreement on a 5-point Likert scale ranging from strongly disagree, disagree, neutral, agree, to strongly agree (step 3). Statements that reached a 75 percent agreement threshold (strongly agree/agree) were adopted as an expert opinion consensus recommendation. Statements where consensus could not be achieved were revised (step 4) and sent out for a second round of ratings (step 5). Revised statements were then adopted if they achieved agreement threshold or left as “consensus could not be achieved” (step 6). This transparent consensus methodology was utilized to then develop this expert guidance given the lack of the availability of any randomized controlled clinical trial data.
2.3. Recommendations

2.3.1. When to Suspect SAMD9/SAMD9L Syndromes

**Hematologic Presentation:**
- SAMD9/SAMD9L should be suspected in patients with chromosome 7 loss [monosomy 7, del(7q), der(1;7)] + bone marrow failure, or MDS, or refractory cytopenia.
- SAMD9/SAMD9L should be suspected in patients with bone marrow failure, MDS, or refractory cytopenia.

**Constitutional Abnormalities:**
- SAMD9/SAMD9L should be suspected in patients with adrenal insufficiency with fetal growth restriction +/- genital phenotypes
- SAMD9/SAMD9L should be suspected in patients with ataxia and cytopenias with or without MRI evidence of cerebellar atrophy.

**Positive Family History**
- SAMD9/SAMD9L should be suspected in patients with a 1st or 2nd degree relative with known SAMD9/SAMD9L

2.3.1.1. Hematologic Presentation

Patients with SAMD9/SAMD9L variants demonstrate hematological phenotype as the common manifestation. The clinical presentation involves single-lineage cytopenia (majorly thrombocytopenia) to pancytopenia with hypocellular marrow and myelodysplastic syndrome (MDS) with monosomy 7 (-7). Progression to advanced leukemic disease (AML, CMML) is rare and had been diagnosed only in a small subset of patients [1, 49, 58, 70]. The main cytogenetic lesion is -7 and depicts a non-random pattern where the chr7 with SAMD9/SAMD9L variant is always lost [47].

The panel recommends SAMD9/SAMD9L aberrations to be suspected in cases with chr7 loss or with a history of -7 that spontaneously disappeared in the setting of BMF, MDS, or refractory...
cytopenia. Additionally, the panel more broadly recommends **SAMD9/SAMD9L** to be suspected in all patients with BMF, MDS, or refractory cytopenia (regardless of cytogenetics).

### 2.3.1.2. Constitutional Abnormalities

Multi-system constitutional anomalies constitute 77% of all cases with **SAMD9/SAMD9L** variants. Patients with MIRAGE depict early onset of adrenal hypoplasia, gonadal dysfunction (both testicular and ovarian dysgenesis), hypospadias, intrauterine growth restriction, gastrointestinal issues (enteropathy, achalasia) with or without urogenital phenotypes. In contrary, neurological findings shows variable age of onset and progression dynamics. While ataxia is specific to **SAMD9L** and has been observed in some cases, cerebellar atrophy and white matter abnormalities has been observed in both **SAMD9/SAMD9L** patient groups [73].

The panel provides the following two recommendations: **SAMD9/SAMD9L** evaluation in children presenting with both adrenal insufficiency and fetal growth restriction with or without urogenital anomalies; and **SAMD9/SAMD9L** be suspected in patients who demonstrate both ataxia and laboratory cytopenia, with or without radiologic evidence of cerebellar atrophy.

### 2.3.1.3. Positive Family History

With the present knowledge, *de novo* **SAMD9** variants are associated with very high penetrance in pedigrees with MIRAGE syndrome, while neurological and hematological disease have exhibited incomplete penetrance. In certain instances, first- and second-degree relatives of an individual with a known **SAMD9/SAMD9L** pathogenic variant can be a carrier and may be at risk of developing phenotype for having the mutation [58]. Hence, the panel recommends these relative to be screened for the **SAMD9/SAMD9L** variant and undergo genetic counseling for further risk evaluation as described below.

**Figure 2: Clinical presentation of **SAMD9/SAMD9L** associated syndromes.**

*Immunologic System:
  - Hypoplastic thymus
  - Viral infections
  - Bacterial infections

*Endocrine System:
  - Fetal growth restriction
  - Failure to Thrive
  - Adrenal hypoplasia/insufficiency
  - Gonadal dysfunction (testicular/ovarian dysgenesis)
  - Ambiguous genitalia
  - 46, XY females

*Hematologic System:
  - Bi-cytopenia/Pancytopenia
  - MDS/AML
  - Chromosome 7 abnormalities

*Neurologic System:
  - Ataxia
  - Cerebellar atrophy/white matter loss
  - Nystagmus
  - Microcephaly
  - Hydrocephalus
  - Polyneuropathy
  - Developmental Delay
  - ADHD

*GI System:
  - Esophageal reflux
  - Achalasia
  - Enteropathy

*Urogenital System:
  - Micropenis
  - Hypospadias
  - Vesicoureteral reflux
  - Nephrotic syndrome

*Family History:
  - History of cytopenias
  - History of prior MDS/AML
  - History of MIRAGE & Ataxia-Pancytopenia syndrome

Presentation ranges from a solitary systemic manifestation to multiple systems involved.
2.3.2. How to Confirm Diagnosis of SAMD9/SAMD9L

Detection of SAMD9/SAMD9L variants

- Diagnostic sequencing of SAMD9/SAMD9L genes should be optimally performed from germline specimens (skin fibroblasts), however hematopoietic specimens (blood/bone marrow) can be used as a first test if enough variant read depth can be delivered to detect germline variants at low allelic frequency.
- SAMD9/SAMD9L mutations identified in hematopoietic specimens should be confirmed for somatic/germline status in skin fibroblasts.
- In patients with clinical phenotype of SAMD9/SAMD9L syndromes but no identified mutation in blood sequencing, testing on skin fibroblasts should be performed.
- All SAMD9/SAMD9L variants identified in patients with a related phenotype should be assessed by a lab familiar with SAMD9/SAMD9L variants since pathogenic mutations have been identified throughout the whole gene and novel mutations are common. Consultation with an expert familiar with the SAMD9/SAMD9L genes may be helpful in variant interpretation.

2.3.2.1. Detection of SAMD9/SAMD9L Variants

With high clinical suspicion for a SAMD9/SAMD9L variant genetic sequencing can confirm the diagnosis. The panel recommends diagnostic sequencing of SAMD9/SAMD9L genes should be optimally performed from germline specimens.

The panel had no consensus on preferred testing platform noting the availability of both targeted gene panels and broader deep sequencing platforms. The non-random loss of chromosome 7 containing the mutated SAMD9/SAMD9L gene copy can make diagnosis a challenge when using hematopoietic specimens, since the variant allele frequency can often be very low, even below 5%. This necessitates germline validation in non-hematopoietic specimens [73].

The panel recommends diagnostic sequencing of SAMD9/SAMD9L genes should be optimally performed from germline specimens. Skin fibroblasts are the preferred non-hematopoietic tissue. Hematopoietic specimens (blood/bone marrow) can be used as a first test if enough variant read depth can be delivered to detect germline variants at low allelic frequency. However, the panel agreed that patients with clinical phenotypes of SAMD9/SAMD9L syndromes without an identified mutation in blood sequencing, should have testing on skin fibroblasts performed. Additionally, the panel recommends SAMD9/SAMD9L variants identified in hematopoietic specimens be confirmed for somatic/germline status in skin fibroblasts.

All SAMD9/SAMD9L variants identified in patients with a related phenotype should be assessed by a lab familiar with SAMD9/SAMD9L variants since pathogenic mutations have been identified throughout the whole gene and novel mutations are common. Consultation with an expert familiar with the SAMD9/SAMD9L genes may be helpful in variant interpretation, since there is currently no clinically commercially available validated functional test to determine if the mutation is pathogenic or not.
2.3.3. Recommended Hematologic Evaluations

| Baseline hematologic evaluation |
|---------------------------------
| • Recommended baseline hematologic evaluation includes complete CBC with differential, reticulocyte count, HbF, and peripheral smear. |

| Baseline bone marrow evaluation |
|---------------------------------
| • Baseline bone marrow evaluation should be performed on all patients with pathogenic/likely pathogenic mutations in the presence of hematologic abnormalities. |
| • Bone marrow evaluation should include assessment of morphology (dysplasia), cellularity, presence of hematopoietic precursors and maturation, cytogenetics (metaphase karyotype and FISH for chromosome 7 q). |

| Detection of cytogenetic abnormalities and acquired somatic mutations |
|---------------------------------------------------------------
| • Somatic testing of myeloid neoplasia mutations (myeloid MDS/AML) panel is recommended for patients with MDS and chromosome 7 loss. |

2.3.3.1. Baseline Hematologic Evaluation

Laboratory presentation of SAMD9/SAMD9L associated syndromes are very heterogeneous ranging from no hematologic abnormalities to pancytopenia. Monocytopenia can be seen in SAMD9L disorders. Full evaluation can help rule in or out other conditions and determine if more invasive testing is necessary. The panel recommends a baseline hematologic evaluation including complete blood count (CBC) with differential, reticulocyte count, hemoglobin electrophoresis, and peripheral smear.

2.3.3.2. Baseline Bone Marrow Evaluation

Baseline bone marrow evaluation should be performed on all patients with pathogenic/likely pathogenic mutations in the presence of hematologic abnormalities. Bone marrow evaluation should include assessment of morphology (dysplasia), cellularity, presence of hematopoietic precursors and maturation, cytogenetics (metaphase karyotype and FISH for chromosome 7 q).

In the case of patients with pathogenic/likely pathogenic or VUS mutations without any hematologic abnormalities, consensus could not be reached on performing an initial bone marrow evaluation. Clinical correlation with assessment and degree of other suggestive phenotypic findings is needed to determine the necessity of bone marrow evaluation on a case-by-case basis.

2.3.3.3. Detection of Cytogenetic Abnormalities and Acquired Somatic Mutations

Leukemic evolution in patients with mutated SAMD9/SAMD9L is not a direct implication of the underlying germline variant but rather an outcome of “compensatory aneuploidy” involving non-random loss of partial or complete chromosome7 (-7) containing the mutated SAMD9/SAMD9L allele. The -7 subsequently function as a (pre) malignant event with a predisposition to acquire additional somatic mutations in known leukemia genes like SETBP1, ASXL1, ETV6, and RAS pathway genes [1, 49, 58, 70]. These clonal mutations further depict high correlations with disease progression, thus identifying them to be leukemic driver events. Current data shows these patients to have a poor prognosis.
When a $\text{SAMD9}/\text{SAMD9L}$ mutation is diagnosed in a patient with MDS and chromosome 7 loss, it is recommended to obtain broad sequencing panel testing for acquired somatic mutations in genes implicated in myeloid neoplasia’s (MDS/AML).

### 2.3.4. Recommended Additional Non-Hematologic Evaluation

<table>
<thead>
<tr>
<th><strong>Baseline immunologic evaluation</strong></th>
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<tbody>
<tr>
<td>Baseline immunologic evaluation should include immunoglobulin panel, T &amp; B &amp; NK cell subset analysis.</td>
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<tr>
<th><strong>Baseline endocrine evaluation</strong></th>
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<tbody>
<tr>
<td>Baseline endocrine evaluation should include growth history, exam for urogenital abnormalities and evaluation for adrenal insufficiency (IGF1, basal AM cortisol with matched ACTH, synacthen, aldosterone and renin activity, gonadotropins) and thyroid function studies.</td>
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<table>
<thead>
<tr>
<th><strong>Baseline neurologic evaluation</strong></th>
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<tbody>
<tr>
<td>Baseline neurologic and neuro-cognitive evaluation should include a comprehensive motor exam.</td>
</tr>
<tr>
<td>A non-contrast brain MRI specifically evaluating for white matter disease (evaluated on T2 weighted images), cerebellar atrophy (evaluated on T1 weighted images) and basal ganglion calcifications should be obtained with documentation of abnormal neurological findings.</td>
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<table>
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<tr>
<th><strong>Upfront HLA typing</strong></th>
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<tbody>
<tr>
<td>Upfront HLA typing of symptomatic patients and their family members should be performed early on in patient’s diagnosis.</td>
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</table>

### 2.3.4.1. Baseline Immunologic Evaluation

$\text{SAMD9}/\text{SAMD9L}$ patients are risk for developing immune dysfunction and severe viral and bacterial infections. This is more commonly seen in syndromic patients. Decreased peripheral B/NK-cells, low IgG/IgM, and increased TNF-alpha and IL-6 levels have been documented in patients with $\text{SAMD9}/\text{SAMD9L}$ variants [73]. Thus, the panel recommends a baseline immunologic evaluation including a quantitative immunoglobulin panel (GAM) and Lymphocyte subset analysis (T, B and NK cell).

### 2.3.4.2. Baseline Endocrine Evaluation

$\text{SAMD9}$ gene encodes a protein involved in growth factor signal transduction, that acts as a growth repressor expressed in endothelial cells and fibroblasts [63]. Mutations within this gene are activating and lead to an antiproliferative effect which leads to a multisystem growth restriction disorder [74]. Expression of $\text{SAMD9}$ mutated proteins cause considerable growth inhibition within cultured cells and fibroblasts [45].

The panel recommends a baseline endocrine evaluation including comprehensive physical exam with documentation of growth including birth weight, any evidence of intrauterine growth restriction, and a urogenital evaluation. Baseline screening for adrenal insufficiency (IGF1, basal AM cortisol with matched ACTH, synacthen, aldosterone and renin activity, gonadotropins) and thyroid function (only accurate following adrenal replacement) are recommended. Usage of a multidisciplinary approach, including an endocrinologist familiar with $\text{SAMD9}/\text{SAMD9L}$ mutations, is important during the initial baseline evaluation to ensure appropriate evaluation is performed.
2.3.4.3. Baseline Neurologic Evaluation

_SAMD9_ and _SAMD9L_ are expressed widely in many tissues and organs, including the CNS. While _SAMD9L_ is more commonly associated with neurologic manifestations than _SAMD9_, there was found to be 30% of _SAMD9_ patients with neurological problems in a large multi-institutional patient cohort [69]. Several cases have also shown phenotypic overlap between the genotypes. Patients with a _SAMD9_ variant have been reported to have hydrocephalus and/or cerebellar hypoplasia with loss of Purkinje and granule neurons [54, 75, 76]. Two other patients have also been reported with post-mortem findings of microcephaly, hydrocephalus and perivascular calcifications with one of those additionally having cerebellar hypoplasia and multifocal polymicrogyria [74].

The panel recommends that all patients diagnosed with a _SAMD9/SAMD9L_ mutation undergo a baseline neurologic and neuro-cognitive evaluation. A well-documented developmental history including documentation of developmental milestones, should be obtained. A comprehensive motor exam including is required.

Providers should consider the utility of neuro imaging at baseline especially if neurologic abnormalities are not present as currently it is unclear if results will change management. Efforts should be made to limit additional anesthesia/sedation risks and delaying evaluation of young patients until they are older or neurologically symptomatic.

With documentation of abnormal neurological findings, the panel recommends a non-contrast brain MRI specifically evaluating for white matter disease (evaluated on T2 weighted images), cerebellar atrophy (evaluated on T1 weighted images) and basal ganglion calcifications be obtained.

As it is necessary for patients with _SAMD9/SAMD9L_ to be evaluated by a multidisciplinary team, it would be reasonable to involve a neurologist familiar with _SAMD9/SAMD9L_ (or MIRAGE syndrome/ataxia-pancytopenia syndrome) during baseline evaluation.

2.3.4.4. Upfront HLA Typing

As the course of disease for patients with _SAMD9/SAMD9L_ is heterogeneous and timeline of progression is unknown, early HSCT may be necessary. There is significant utility of knowing upfront donor status if rapid progression to HSCT is required, thus the panel recommends upfront HLA typing of symptomatic patients and their family members be performed early on in patients diagnosis.
2.3.5. Management of Patients with Pathogenic SAMD9/SAMD9L Germline Mutations by Phenotype

<table>
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<th>Asymptomatic</th>
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<tr>
<td>Yearly CBC evaluation.</td>
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<tr>
<td>If patient develops new neurologic symptoms than repeat brain imaging would be warranted.</td>
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<tr>
<td>All new symptoms should be evaluated with a multidisciplinary approach</td>
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<tr>
<th>Mild Hematologic/Immunologic</th>
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<tr>
<td>CBC at least every 3 months.</td>
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<tr>
<td>Repeat bone marrow evaluation recommended every 12 months, unless patient develops worsening cytopenias then repeat bone marrow evaluation would be warranted sooner.</td>
</tr>
<tr>
<td>If patient develops new neurologic symptoms than repeat brain imaging would be warranted.</td>
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<tr>
<td>All new symptoms should be evaluated with a multidisciplinary approach</td>
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<tr>
<th>Intermediate Hematologic/Immunologic</th>
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<tr>
<td>If patient develops new neurologic symptoms than repeat brain imaging would be warranted.</td>
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<td>All new symptoms should be evaluated with a multidisciplinary approach</td>
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<tr>
<th>Severe Hematologic/Immunologic</th>
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<tr>
<td>We recommend these patients proceed immediately to bone marrow transplant as soon as an acceptable donor is acquired and infections adequately controlled.</td>
</tr>
<tr>
<td>All potential familial stem cell donors must be adequately screened to ensure they are not SAMD9/SAMD9L mutation carriers prior to proceeding as donor.</td>
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Table 1: SAMD9/SAMD9L Germline Mutational Phenotype Definitions

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<tr>
<th>Asymptomatic</th>
<th>SAMD9/SAMD9L mutation carrier without any evidence of current cytopenias or hematologic disease</th>
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<tbody>
<tr>
<td>Mild Hematologic/Immunologic</td>
<td>SAMD9/SAMD9L mutation carrier with evidence of current cytopenias. NO: excessive blasts, monosomy 7, acquired clonal abnormalities (except UPD7 clone/somatic SAMD9/SAMD9L mutation). Patient cannot be transfusion dependent, have ANC &lt; 500 or any serious infections</td>
</tr>
<tr>
<td>Intermediate Hematologic/Immunologic</td>
<td>SAMD9/SAMD9L mutation carrier with evidence of current cytopenias and monosomy 7. NO: excessive blasts, additional acquired clonal abnormalities (except UPD7 clone/somatic SAMD9/SAMD9L mutation). Patient cannot be transfusion dependent, have ANC &lt; 500 or any serious infections</td>
</tr>
<tr>
<td>Severe Hematologic/Immunologic</td>
<td>SAMD9/SAMD9L mutation carrier with evidence of current cytopenias and excessive blasts, monosomy 7 and acquired clonal abnormalities (except UPD7 clone/somatic SAMD9/SAMD9L mutation) OR patient is transfusion dependent, has ANC &lt; 500 or any serious infection</td>
</tr>
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2.3.5.1. Asymptomatic

We defined an asymptomatic patient as a SAMD9/SAMD9L variant carrier without evidence of cytopenias or hematologic disease. Although asymptomatic carriers were recommended to undergo initial diagnostic evaluation as outlined above, the panel could not achieve consensus on the necessity of performing a baseline bone marrow evaluation without hematologic abnormality. Panel members cited a lack of current evidence on the natural clinical disease course to perform
a sedated procedure on an asymptomatic carrier. Patients should have an annual evaluation with a hematologist oncologist familiar with MDS and bone marrow failure. Evaluation should include comprehensive physical exam. Recommended laboratory screen should include peripheral complete blood count. These disorders are associated with dynamic change over time and if cytopenias are noted than patients progress to the next phenotypic category as described below and will require a more intensive work-up.

Non-hematologic surveillance should be determined by clinical findings on baseline evaluation including appropriate surveillance and a multidisciplinary subspecialty follow up approach. Any development of neurologic symptoms warrants initial or repeat brain MRI. Any new symptoms warrant appropriate sub-specialist evaluation.

2.3.5.2. Mild Hematologic/Immunologic

We defined a mild hematologic/immunologic patient as a $\text{SAMD9}/\text{SAMD9L}$ variant carrier with evidence of current cytopenias. Patients cannot have excessive blasts, monosomy 7, or acquired clonal abnormalities (except UPD7 clone /somatic $\text{SAMD9}/\text{SAMD9L}$ mutation). Patients cannot be transfusion dependent, have ANC < 500 or any serious infections.

The panel agreed that this sub-group of patients require more frequent monitoring, recommending CBC’s at least every 3-months and repeat bone marrow evaluation every 12-months. There should be a low threshold to pursue repeat bone marrow aspirate and biopsy with any notable change in peripheral blood counts. Bone marrow evaluation should include cytogenetics using metaphase G-banding, karyotyping and FISH for Chr 7 abnormalities and FISH MDS panel, and aspirate should be sent for sequencing panel monitoring for acquisition of somatic driver mutations.

Non-hematologic surveillance should be determined by clinical findings on baseline evaluation including appropriate surveillance and a multidisciplinary subspecialty follow up approach. Any development of neurologic symptoms warrants initial or repeat brain MRI. Any new symptoms warrant appropriate sub-specialist evaluation.

If cytopenias resolve in this phenotype population it is acceptable to follow recommendations for the asymptomatic category and decrease frequency of monitoring.

2.3.5.3. Intermediate Hematologic/Immunologic

We defined an intermediate hematologic/immunologic patient as a $\text{SAMD9}/\text{SAMD9L}$ variant with evidence of current cytopenias and a cytogenetic abnormality of chromosome 7 (including monosomy 7) without excessive blasts, or additional acquired clonal abnormalities (except UPD7 clone/somatic $\text{SAMD9}/\text{SAMD9L}$ mutation). Additionally, patients cannot be transfusion dependent, have ANC < 500, or any serious infections.

Although supportive care in the form of blood transfusions and antibiotics for infections can ameliorate disease, allogenic hematopoietic stem cell transplant (HSCT) represents the only curative option for a patient with $\text{SAMD9}/\text{SAMD9L}$ with progression to MDS [64]. In other disease processes cytopenias and monosomy 7 automatically indicate a malignant process, however as we have discussed the ‘compensatory aneuploidy’ seen in patients with $\text{SAMD9}/\text{SAMD9L}$ variants is presumably adaptive and thought to allow for more rapid cellular
growth. However, when the progenitor system fails to select benign revertant clones, than the created clones predispose to MDS [77, 78].

Bone marrow transplant outcomes in patients with \textit{SAMD9/SAMD9L} generally are favorable, noting overall survival around 85% for all documented patients who have undergone HSCT [1, 48, 60, 64, 65, 77]. This is consistent with the overall post-transplant survival rates for all conditions [79]. Poor outcomes were typically associated with MIRAGE syndrome phenotype. Outcome was also influenced by karyotype, where -7 patients were noted to have worse outcomes in comparison to patients with a normal karyotype, irrespective of the underlying genotype [80]. There has also been a high rate of ongoing medical issues observed in MIRAGE syndrome transplant survivors, including adrenocortical insufficiency, diarrhea, need for supplemental nutrition, and developmental delays, however most of these issues are related to pre-existing MIRAGE syndrome manifestations [64].

The panel carried out a lengthy debate regarding the indication for HSCT in \textit{SAMD9} and \textit{SAMD9L} patients with evidence of cytopenias and a clonal abnormality of chromosome 7. The panel could not achieve consensus for this sub-group of patients regarding recommended upfront therapeutic HSCT. Some providers felt that for patients with stable disease that are not transfusion dependent that the addition of a clonal monosomy 7 was not an indication for immediate transplant. Others felt that presents of monosomy 7 should indicate urgent HSCT. Most providers felt that age was a major factor in considering immediate bone marrow transplant and felt that older patients were at increased risk and should have a lower threshold for transplant. Currently more data will need to be collected to come up with an expert consensus and recommendation.

It was agreed upon however, that regardless patients who develop transfusion dependence, increase in blasts or serious infection progress to the severe phenotypic definition and should follow the recommendations described below.

There was also discussion of proceeding directly to transplant in patients who develop a secondary clonal oncogenic mutation. If not previously performed, it is important to obtain testing to ensure clonal progression is identified as early as possible. It has been observed in several patient cohorts [1, 58, 70] that these patients have a worse prognosis and while this still requires continued study, and there is not an agreed upon consensus at this time, it was generally felt that these patients would benefit from immediate bone marrow transplant.

\textbf{2.3.5.4. Severe Hematologic/Immunologic}

We defined a severe hematologic/immunologic phenotype as a \textit{SAMD9/SAMD9L} variant carrier with evidence of current cytopenias and excessive blasts, monosomy 7 and acquired clonal abnormalities (except UPD7 clone/somatic \textit{SAMD9/SAMD9L} mutation) OR patient is transfusion dependent, has ANC < 500, or any serious infection. We recommend these patients proceed immediately to bone marrow transplant as soon as an acceptable donor is acquired, and infections are adequately controlled.

All potential familial stem cell donors must be adequately screened to ensure they are not \textit{SAMD9/SAMD9L} mutation carriers prior to proceeding as donor. There is no recommended preparative/immunosuppressive regiment prior to bone marrow transplant and facilities should follow their specific institutional protocols for patients with myelodysplastic syndrome.
2.3.6. Additional General Recommendations

**Research Studies**
- Patient's with SAMD9/SAMD9L variants should be offered to participate in research studies and if consent obtained biomaterial should be banked.

**Genetic Counseling**
- Genetic counseling needs to be incorporated in all patients/families found to have a SAMD9/SAMD9L germline mutation in addition to appropriate psychological support to help families understand and cope with their diagnosis.
- Genetic testing should be offered to all first-, second- and third-degree relatives of an individual with a SAMD9/SAMD9L mutation to ensure appropriate surveillance of all potential mutation carriers.

**Infectious Prophylaxis**
- Infectious prophylaxis should follow local hospital policy, no specific recommendations are currently in place.

2.3.6.1. Research Studies

Increased data collection is dependent upon identification of patients with SAMD9/SAMD9L germline mutations, and the enrollment of these patients on studies and patient registries to observe the natural evolution of this disease. The goals of these registries are to estimate cumulative incidence and determine medical complications in these patients both with and without treatment. With this data we will continue to formalize safe and effective treatments for patients with SAMD9/SAMD9L mutations. These studies will also serve as a source for collection of data and bio-specimens for future hypothesis driven trials as questions arise. It is important to
offer all patient's with \textit{SAMD9/SAMD9L} variants participation in research studies. If consent is obtained biomaterial should also be banked.

\subsection*{2.3.6.2. Genetic Counseling}

Genetic counseling needs to be incorporated in all patients and families found to have a \textit{SAMD9/SAMD9L} germline mutation to discuss the benefits, risks, and limitations of genetic testing for familial mutations in addition to appropriate psychological support to help families understand and cope with their diagnosis. Genetic testing should be offered to all first-, second-, and third-degree relatives of an individual with a \textit{SAMD9/SAMD9L} mutation to ensure appropriate surveillance of all potential mutation carriers. Many genetic counselors do not know much about this rare condition so ideally patients should be referred to a center where genetic counselors are familiar with \textit{SAMD9/SAMD9L} mutations and will be better able to appropriately counsel families. Family members that are positive for the genetic mutation should also be offered enrollment in research studies and databases.

\subsection*{2.3.6.3. Infectious Prophylaxis}

Infectious prophylaxis should follow local hospital policy, no specific recommendations are currently in place.

\subsection*{2.4. Conclusions}

This manuscript presents recommendations derived from a consensus driven \textit{SAMD9/SAMD9L} symposium that was the joint effort of experts from multiple international medical and basic science specialists and follow up anonymous surveys to ensure an appropriate consensus was achieved on each individual statement.

While these consensus statements help to provide guidelines for initial evaluation and therapy there is still a lot of data needed to help formalize standards of care for precises disease management.

Standard of care currently is for all patient who develop a somatic monosomy 7 to undergo bone marrow transplantation, however new data is becoming available that shows patients undergo a spontaneous regression of the monosomy 7 mutation or a clonal evaluation that could keep patients from needing to undergo invasive and potentially harmful procedures.

Increased data collection is dependent upon identification of patients with \textit{SAMD9/SAMD9L} germline mutations, and then creation of a prospective registry to observe the natural evolution of this disease.
Chapter 3: Discussion

3.1. Rare Diseases

Rare diseases, otherwise known as neglected or orphan diseases, are generally defined as a health condition that only affects a small number of the population. The exact percentage of the population affected varies based on the defining entity, with the United States using the cutoff of 200,000 persons affected and the European Union defining a rare disease as affecting fewer than 5 per 10,000 persons [81]. These diseases are generally chronic, progressive, life threatening or debilitating, with no curative or effective treatment for most of them [82, 83].

Rare diseases have a genetic origin in up to 80% of cases, resulting from dysfunction of a specific pathway usually due to defective gene expression or protein production [83]. Children represent 50-70% of these patients, with 30% dying before reaching the age of five [82]. There are between 6,000 and 8,000 rare diseases identified, so while they are described as rare they still affect approximately 8% of the population [83].

Management of rare diseases becomes difficult secondary to a number of factors including: geographical dispersion of patients and disease specialists, limited number of specialists, and limited information and knowledge [84]. In addition, prior to 1983, there was not an incentive for commercial drug companies to develop drugs for rare diseases. The Orphan Drug Act was signed in the US by President Regan which helped to promote orphan drug development by giving tax incentives, market exclusivity, and user fee exemptions to companies developing drugs and therapies for orphan and rare diseases [81, 83]. This reduced research and development costs, which enabled larger pharmaceutical companies to focus on drug development for rare diseases, however this still requires there to be both a diagnosis and information available about these diseases for effective drug production and management to take place.

Diagnosis of rare diseases has rapidly expanded as genetic testing has become more readily available. Technological advances and reduction of costs associated with genetic testing has enabled the use of precise genetic methods, including single-nucleotide polymorphism (SNP) genotyping and exome sequencing, on a much broader patient population [83].

Successful management and drug development for rare diseases is still limited by an incomplete knowledge base of the underlying disease mechanisms and clinical endpoints, as information is limited by small sample size of comparable patients for clinical trials [81]. This creates a need for development of a shared network of information linking all patients with the same or similar diagnosis together.

3.2. Importance of Patient Registries for Rare Diseases:

Many of the challenges with management of rare diseases can be improved by creation of an integrated information system for rare diseases. It is important for this information system to be well-developed and have the appropriate methods for data collection, storage, processing, retrieval, and sharing [82]. Registry databases and tissue repositories are an essential tool for providing important data to a large spectrum of researchers and clinicians [83]. Rare disease registries pose some additional challenges over the basic concepts around registry planning, design, and implementation for standard disease registries. Clinicians with enough expertise and
direct exposure for managing these patients are more limited, which requires a larger outreach effort to identify and recruit enough patients in order to properly understand the epidemiology and natural history of the disease [81].

The scope and objectives of rare disease registries are often broader than a typical disease registry because of knowledge gaps within these specific diseases including the absence of standards of care or treatment guidelines, the use of experimental or adjunctive therapies, and a poor understanding of how these diseases should be monitored. It is important for there to be a balance ensuring that rare disease registries are comprehensive enough to address critical questions, while not being so extensive that the data cannot be acquired and maintained reasonably [81].

“A well-designed registry provides an infrastructure that can support different needs in an efficient way and eliminate barriers to scientific progress [81].” The goals of a rare disease registry typically include: 1) to connect affected patients, families, and clinicians; 2) to learn the natural history, evolution, risk, and outcomes of specific diseases; 3) to support research on genetic, molecular, and physiological basis of rare diseases; 4) to establish a patient base for evaluation drugs, medical devices, and orphan products.

There is generally limited available literature on rare diseases and most clinicians have not seen a wide spectrum of patients with rare diseases. Registries can provide a connection to essential information and a way for disease experts to disseminate information to providers to assist with advising and counseling patients [81].

In addition to connecting clinicians, registries can provide affected patients and families with a multitude of support services. The network created by the registry can enable patients and families to gain a deeper understanding of their condition through a common location or forum to disseminate information about the disease. The registry can also provide a means for connecting patients to other patients with the same disease. These connections provide not only a psychological support system for those patients and their families but can also help to deepen a patients understanding of the disease by having someone else to discuss their condition with.

Rare diseases are often described generally by their symptoms at time of diagnosis. As diagnostic techniques are refined these disease descriptions can be expanded and are better described. Registries can be used to help develop a better understanding of disease burden, progression, genotypic and phenotypic heterogeneity, and potential endpoints. Rare disease registries and natural history studies, often from multiple centers, will help extract stronger and more generalizable safety, diagnostic, and prognostic information to assist with better defining these diseases [81].

Biorepositories are important to understanding rare diseases at the genetic, molecular, and cellular level. Having a meaningful biorepository for a rare disease requires collection of materials from a sufficient population to allow for generalization about the fundamental features and disease diversity at this level. Registries are an important compliment to biorepositories and add significant strength to the data as they incorporate both longitudinal clinical and phenotypical data with the genetic, molecular, and cellular biology [81].

Clinical development for drugs for effective treatment for rare diseases can be challenging due to low prevalence and phenotypic heterogeneity. There is limited opportunity for randomized
controlled clinical trials and often the disease course is not sufficiently known, making clinical trial design difficult for new drugs [85]. Rare disease registries can be an invaluable source of information to enable studies to provide useful drugs or devices. The knowledge gathered from rare disease registries, including prevalence, clinical disease course, prognostic subgroups, and endpoints, can provide the foundation for developing clinical trial design. Registries can contribute information to estimate sample size for a new trial. This can alleviate the need for pilot studies [85]. Rare disease registries can also be used as the platform for case records, data collection, randomization, and follow-up, alleviating some financial and logistical concerns when starting a randomized control trial. In some cases, where severely progressive diseases have no treatment available, the rare disease registry could serve as historical controls for a single arm trial [85].

Using rare disease registries in the post-marketing phase of drug development can also assist with systemic collection of real-world data on new therapies. Registries can provide evidence to clear any uncertainty on effectiveness and safety of a product in conditions of routine clinical practice and in a wider patient population [85].

3.3. Future Steps for SAMD9/SAMD9L Research:

The current number of patients with SAMD9 and SAMD9L mutations in the United States is 200 to 300, classifying it as a rare disease. The consensus statements discussed within the body of this thesis help to provide guidelines for initial evaluation and therapy for patients with SAMD9 and SAMD9L variants, however there is still a need for increased data to formalize standards of care for precise disease management.

As an integral part of my research, I helped to develop a bone marrow failure registry that will contain all bone marrow failure patients at St. Jude, including those with SAMD9/SAMD9L variants. This registry was developed by creation of a database utilizing REDCap Cloud. This database includes both retrospective patients and the prospective cohort that is the prospective natural history study of patients with SAMD9 and SAMD9L variants. Data variables for this registry are described in Table 2. This study is now currently open and enrolling patients at St. Jude. The goal of this is to estimate cumulative incidence and determine medical complications in these patients both with and without treatment. With this data we can then identify both safe and effective treatments for these patients by offering necessary life-saving therapy while minimizing risky interventions. This study will also serve as a source for collection of data and bio-specimens for future hypothesis driven trials as questions arise.

In addition to the natural history study, St. Jude has developed a mutational database of both published and unreported SAMD9 and SAMD9L mutations. Because these mutations are so new and heterogeneous, they are difficult to understand. Having a location that can provide an expert opinion on these mutations and give second opinions on these genetic testing results is essential [86].
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<th>Data Form</th>
<th>Data Collected</th>
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<td><strong>Demographics</strong></td>
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<td>• Ethnic Category</td>
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<td>• Biospecimen Available</td>
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<td>• Other Study Enrollment</td>
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<td>• Maternal History of Miscarriages</td>
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<td>• Mode of Conception</td>
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<td><strong>Medical History</strong></td>
<td>• History of Prior Cytopenias</td>
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<td>• Any Other Medical Problems</td>
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<td>• Primary Modality of Diagnosis</td>
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<td><strong>Complete Blood Count</strong></td>
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References:

58. Wong, J.C., et al., Germline SAMD9 and SAMD9L mutations are associated with extensive genetic evolution and diverse hematologic outcomes. JCI Insight, 2018. 3(14).


