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Outcomes of Hematopoietic Cell Transplantation in Patients with Germline *SAMD9/SAMD9L* Mutations

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A B S T R A C T

Germline mutations in *SAMD9* and *SAMD9L* genes cause MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) (OMIM: *610456) and ataxia-pancytopenia (OMIM: *611170) syndromes, respectively, and are associated with chromosome 7 deletions, myelodysplastic syndrome (MDS), and bone marrow failure. In this retrospective series, we report outcomes of allogeneic hematopoietic cell transplantation (HCT) in patients with hematologic disorders associated with *SAMD9/SAMD9L* mutations. Twelve patients underwent allogeneic HCT for MDS (n = 10), congenital amegakaryocytic thrombocytopenia (n = 1), and dyskeratosis congenita (n = 1). Exome sequencing revealed heterozygous mutations in *SAMD9* (n = 6) or *SAMD9L* (n = 6) genes. Four *SAMD9* patients had features of MIRAGE syndrome. Median age at HCT was 2.8 years (range, 1.2 to 12.8 years). Conditioning was myeloablative in 9 cases and reduced intensity in 3 cases. Syndrome-related comorbidities (diarrhea, infections, adrenal insufficiency, malnutrition, and electrolyte imbalance) were present in MIRAGE syndrome cases. One patient with a familial *SAMD9L* mutation, MDS, and morbid obesity failed to engraft and died of refractory acute myeloid leukemia. The other 11 patients achieved neutrophil engraftment. Acute post-transplant course was complicated by syndrome-related comorbidities in MIRAGE cases. A patient with *SAMD9L*-associated MDS died of diffuse alveolar hemorrhage. The other 10 patients had resolution of hematologic disorder and sustained peripheral blood donor chimerism. Ten of 12 patients were alive with a median follow-up of 3.1 years (range, 0.1 to 14.7 years). More data are needed to refine transplant approaches in *SAMD9/SAMD9L* patients with significant comorbidities and to develop guidelines for their long-term follow-up.

INTRODUCTION

In recent years, advances in genetic interrogation of patient samples have led to discovery of several novel genes that underlie inherited bone marrow failure and myelodysplastic syndrome (MDS) [1]. These include *SAMD9* (sterile α-motif domain-containing protein 9) and *SAMD9L* (*SAMD9-like*) genes, located head to tail on chromosome 7q21.2 in a region that is frequently deleted in myeloid malignancies [2,3].

Germline mutations in *SAMD9* and *SAMD9L* cause the multisystem disorders, MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy) and ataxia-pancytopenia syndromes, respectively [4-6]. Recent studies in children reported a rate of *SAMD9* and *SAMD9L* mutations in 18.6% and 17% cases with suspected inherited bone marrow failure syndromes and those with primary MDS, respectively [7,8].

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SAMD9 and SAMD9L proteins are involved in endosomal trafficking and negatively regulate cell proliferation [9]. Gain-of-function heterozygous mutations in these genes lead to cellular growth restriction and hypoplasia, resulting in cytopenias, bone marrow failure, and immunodeficiency. Interestingly, in many cases, there is a nonrandom loss of the mutated allele via full or partial deletion of chromosome 7 [4,10-12]. The resultant monosomy 7 or deletion 7q can result in the development of MDS and acute myeloid leukemia (AML) [8,11,12]. Conversely, other "genetic correction" events such as in *cis* missense, nonsense, or loss of heterozygosity through uniparental disomy can result in normal hematopoiesis.

Since the initial report of MIRAGE syndrome in 2016, a series of studies has described clinical and genetic findings in patients and families with *SAMD9/SAMD9L* mutations [7,11,13]. Hematopoietic cell transplantation (HCT) therapy has been included in some reports, but transplantation details are lacking. A recent article by Sarthy et al. [14] documented 2 children with MIRAGE syndrome who succumbed to post-transplant complications due to syndrome-related comorbidities. We aimed to obtain a more complete assessment of transplant outcomes and the challenges and complications encountered in these patients.

METHODS

After management of 2 cases with MIRAGE syndrome, additional cases were identified by literature search and peer consultations. For inclusion, patients were required to have a confirmed heterozygous mutation in the *SAMD9* or *SAMD9L* gene and a minimum of 1-year follow-up post-transplant. Deidentified data for each case were collected by using a standardized questionnaire. All studies involving human subjects were performed in accordance with site-specific protocols approved by the institutional review board and in accordance with Declaration of Helsinki guidelines.

The primary study endpoints were overall survival and event-free survival. Safety and tolerability of HCT and impact of pretransplant comorbidities were evaluated by occurrence and severity of post-transplant complications, need for life support measures, and risk of transplant-related mortality. Transplant outcomes were defined using Center for International Blood and Marrow Transplant Research criteria [15]. Grading of acute graft-versus-host disease (GVHD) and diagnosis of chronic GVHD were based on standard criteria [16]. Surviving patients were censored at last follow-up. Continuous variables were summarized as median and range of values and analyzed using the Mann-Whitney test. Survival curves were generated using the Kaplan-Meier method and compared using the log-rank (Mantel-Cox) test using the GraphPad Prism 7 software (GraphPad Software, San Diego, CA).

RESULTS

Twelve patients underwent allogeneic HCT for hematologic disorders associated with germline *SAMD9* (n = 6) or *SAMD9L* (n = 6) mutations (Table 1). Patients 3, 4, 6, 11, and 12 (Table 1) were included in previous reports [11,13,17]. Indication for transplant was MDS in 10 of 12 (83%) cases. One *SAMD9* patient with markedly reduced megakaryocytic precursors in marrow underwent transplantation for a presumed diagnosis of congenital amegakaryocytic thrombocytopenia, and 1 patient with *SAMD9L* mutation and shortened telomeres underwent transplantation on a presumed diagnosis of dyskeratosis congenita.

Median age at presentation for patients with *SAMD9* mutations (1.65 years; range, 0.17 to 4.8 years) was similar to those with *SAMD9L* mutations (1.43 years; range, 0.67 to 12.6 years). Six patients had pancytopenia, including 5 with thrombocytopenia and 1 with anemia. Bone marrow was hypocellular in 11 (92%) cases and showed dysplasia most prominently in the megakaryocytic lineage in most cases. Chromosome 7 abnormalities, including monosomy 7 and chromosome 7q deletions, were present in all cases. All except 1 case showed somatic mosaicism for chromosome 7 abnormalities (ie, detection of a monosomy 7 or chromosome 7 deletion clone in only a fraction of hematopoietic cells in bone marrow).

Exome sequencing revealed 5 different missense heterozygous mutations in the 6 *SAMD9* cases and 4 different missense mutations in the 6 *SAMD9L* cases. Their genomic details and pathogenicity assessment of variants are summarized in Table 2 and cross-referenced [5,7,8,12,13,17-20]. Six of 12 cases were familial. Four *SAMD9* patients had phenotypic features of MIRAGE syndrome (patients 1, 2, 5, and 6; Tables 1 and 2); unique findings included panhypopituitarism, laryngeal cleft, and glomerulosclerosis. Two other cases with a *SAMD9* mutation had milder phenotypes with growth restriction in 1 and hypospadias and a bifid scrotum in another. The remaining patients had no phenotypic abnormalities.

Transplant details of individual cases are summarized in Table 3. Median age at HCT was 2.8 years (range, 1.16 to 12.8 years). Median age at HCT tended to be higher in *SAMD9* patients versus *SAMD9L* patients at 4.15 years versus 2.2 years, respectively ($P = .81$). Median time from initial presentation to transplant was 0.45 years (range, 0.2 to 6.53 years). There was an interval of 5.5 and 6.53 years from initial diagnosis to HCT in 2 cases of MIRAGE syndrome because in these cases, blood counts seemed to show improvement before patients developed sustained marrow failure. Stem cell sources included bone marrow (matched unrelated, n = 7; HLA identical sibling, n = 2; and haploidentical parent, n = 1) and unrelated cord blood (n = 2). Nine patients received myeloablative conditioning (busulfan based, n = 7, or total-body irradiation based, n = 2). Three patients received reduced-intensity conditioning with fludarabine, cyclophosphamide, or melphalan, with rabbit antithymocyte globulin or alemtuzumab.

Clinically significant pretransplant comorbidities were present in *SAMD9* cases with MIRAGE syndrome (Table 3). These included chronic diarrhea, electrolyte imbalance, infections, adrenal insufficiency, failure to thrive, lung disease, and renal dysfunction. One patient with *SAMD9L* mutation (patient 10, Table 2 and Table 3) had been treated for hemophagocytic lymphohistiocytosis, disseminated sepsis, invasive fungal infections before transplant.

Post-transplant complications included pericardial effusions (n = 3), veno-occlusive disease of liver (n = 3), thrombotic microangiopathy (n = 2), and diffuse alveolar hemorrhage (n = 1). Unique complications in several MIRAGE syndrome cases included large volume stool losses with dehydration and electrolyte imbalance, temperature and blood pressure instability, and hypoxia. Eight patients required transfer to intensive care for management of respiratory failure (n = 5), sepsis (n = 1), and severe hypertension (n = 1) and VOD of liver (n = 1).

One patient with a familial *SAMD9L* mutation, MDS, (patient 7, Table 3) and morbid obesity failed to engraft following reduced-intensity conditioning with double unrelated cord blood transplantation. All other patients achieved neutrophil and platelet engraftment at a median of 16 days (range, 12 to 19; n = 11) and 17 days (range, 12 to 40; n = 10) post-HCT, respectively. Two patients developed grade II to III acute GVHD, which resolved with treatment. Two patients developed mild skin chronic GVHD. Two patients have chronic lung disease, and 2 other patients have chronic kidney disease. One patient with *SAMD9L* mutation and MDS (patient 7, Table 3) with failed engraftment subsequently developed AML and died of its treatment complications. A second patient, with *SAMD9L* mutation and MDS (patient 10, Table 3), died of diffuse alveolar hemorrhage while receiving defibrotide for treatment of veno-occlusive disease of liver. Immune reconstitution data are summarized in Table 4.

Ten of 12 patients were alive with a median follow-up of 3.1 years (range, 0.1 to 14.7 years). All surviving patients (n = 10) at time of last follow-up had resolution of hematologic disorder,

Table 1
Patient Characteristics

Patient No.	1	2	3	4	5
Age at initial presentation, years	0.17	1	3.1	4.8	0.8
Gender	M	M	F	M	F
Race/Ethnicity	Hispanic	Caucasian	Caucasian	Caucasian	African American
Gene mutation	*SAMD9* c.2471G>A; pR824Q	*SAMD9* c.4690G>A; p.G1564S	*SAMD9* c.3406G>C; p.E1136Q	*SAMD9* c.3406G>C p.E1136Q	*SAMD9* c.2407 G>C; p.E803Q
Family member with same gene mutation	Parents negative	Parents negative	Patient no. 3 and 4 in this report, a younger sibling and their mother positive	Patient no. 3 and 4 in this report, a younger sibling and their mother positive	Parents negative
MIRAGE syndrome features (*SAMD9* cases)	Infections, restriction of growth, adrenal, genital, enteropathy	MDS, infections, restriction of growth, adrenal, enteropathy	MDS	MDS, genital	MDS, infections, restriction of growth, enteropathy
Other clinical findings	Newborn Period: Born at 29 weeks, birth weight 982 grams, mechanical ventilation. Chronic lung disease of prematurity. Microcephaly, developmental delay, panhypopituitarism, laryngeal cleft, intussusception, FSGS	Newborn Period: Born at 34 weeks, birth weight 1425 grams, no mechanical ventilation. Achalasia of esophagus, developmental delay	–	–	Newborn Period: Born at 36 weeks, birth weight 1895 grams, no mechanical ventilation. Staphylococcal sepsis with respiratory failure. Developmental delay
Hematology	Thrombocytopenia followed by pancytopenia. Hypocellular marrow, megakaryocytic hypoplasia	Pancytopenia. Hypocellular marrow, reduced megakaryocytes and dysplasia	Thrombocytopenia. Hypocellular marrow, trilineage dysplasia	Hypocellular marrow, trilineage dysplasia, refractory cytopenia of childhood	Pancytopenia. Normocellular marrow, megakaryocytic dysplasia
Chromosome 7	Somatic mosaic monosomy 7, somatic mosaic chr. 7q deletion, UPD chr. 7	Somatic mosaic monosomy 7, somatic mosaic 7q31 deletion, UPD chr. 7	Monosomy 7	Somatic mosaic monosomy 7	Somatic mosaic monosomy 7
Patient No.	6	7	8	9	10
Age at initial presentation, years	2.3	12.6	0.9	8.1	0.7
Gender	M	F	M	F	M
Race/Ethnicity	Caucasian	Hispanic	Hispanic	Caucasian	African American
Gene mutation	*SAMD9* c.2318T>C; p.I773T	*SAMD9L* c.1877C>T; p.S626L	*SAMD9L* c.1877C>T; p.S626L	*SAMD9L* c.3538T>C; p.W1180R	*SAMD9L* c.4651 G>C; p.V1551L
Family member with same gene mutation	Mother negative, father unavailable	Patients no. 7 and 8 in this report are nephews. Parents not tested. A maternal aunt is positive	Patients no. 7 and 8 in this report are nephews. Parents not tested. A maternal aunt is positive	Parents not tested	Parents negative
MIRAGE syndrome features (*SAMD9* cases)	MDS, infections, restriction of growth, adrenal, genital, enteropathy	N.A.	N.A.	N.A.	N.A.
Other clinical findings	Newborn Period: Born at 34 weeks, birth weight 1853 grams, no mechanical ventilation. FSGS, short telomeres. microcephaly, hypotelorism, strabismus, beaked nose, reactive airway disease, warts	–	–	Hypogammaglobulinemia	HLH. Sepsis
Hematology	Thrombocytopenia. Hypocellular marrow, dysplastic megakaryocytes	Hypocellular marrow, dyserythropoiesis	Hypocellular marrow, dyserythropoiesis and dysmegakaryopoiesis	Hypocellular marrow, atypical megakaryocytes	Pancytopenia. Hypocellular marrow, dyserythropoiesis, dysgranulopoiesis
Chromosome 7	Mosaic chr. 7q deletion	Absence of heterozygosity chr. 7q (myeloid)	Mosaic monosomy 7	Mosaic monosomy 7	Mosaic monosomy 7

(*continued*)

Table 1 (*Continued*)

Patient No.	11	12					
Age at initial presentation, years	1.6	1.3					
Gender	F	M					
Race/Ethnicity	Caucasian	Caucasian					
Gene mutation	*SAMD9L* c.2957G>A; p.R986H	*SAMD9L* c.2957G>A; p.R986H					
Family member with same gene mutation	Patients no. 11 and 12 in this report are siblings. Father positive. Mother negative	Patients no. 11 and 12 in this report are siblings. Father positive. Mother negative					
MIRAGE syndrome features (*SAMD9* cases)	N.A.	N.A.					
Other clinical findings	Eczema	Eczema					
Hematology	Thrombocytopenia followed by pancytopenia. Normocellular marrow with dysplasia	Pancytopenia. Normocellular marrow with megakaryocyte dysplasia					
Chromosome 7	Mosaic monosomy 7	Mosaic monosomy 7, mosaic chr. 7q deletion					

Abbreviations: Chr. 7 (chromosome 7); FSGS (Focal sclerosing glomerulosclerosis); HCT (hematopoietic cell transplantation); HLH (hemophagocytic lymphohistiocytosis); MDS (myelodysplastic syndrome); MIRAGE syndrome (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy); and, UPD (uniparental disomy)

had no chromosome 7 abnormalities, and sustained peripheral blood donor chimerism (90% to 100%). All patients were thriving. *SAMD9* cases had varying degrees of developmental delays (n = 6) and chronic kidney disease (n = 3). All patients with clinical characteristics of MIRAGE syndrome (n = 4) were short for age, required supplemental feeds, and had persistent adrenal insufficiency. In *SAMD9L* cases (n = 4), no clinical neurologic manifestations have been observed so far.

DISCUSSION

In this report, we describe transplant details and outcomes in a series of patients with hematologic diseases associated with *SAMD9/SAMD9L* germline mutations. We found that most patients underwent transplantation for MDS with chromosome 7 abnormalities and received myeloablative conditioning with HCT from nonsibling donor graft sources. Allogeneic HCT led to successful resolution of MDS or marrow failure, with sustained donor chimerism and excellent survival.

On review of literature, we found 10 other cases with *SAMD9* mutation who underwent HCT. A 4-year-old child with MIRAGE syndrome and monosomy 7 MDS underwent transplantation with active AML and died of Epstein-Barr virus-related lymphoproliferative disorder a year later [4]. Wilson and colleagues [21] reported a patient with MIRAGE syndrome who underwent reduced-intensity conditioning and unrelated donor HCT that led to resolution of monosomy 7 MDS. Sarthy et al. [14] described a patient with marrow failure and another patient with MDS who had severe MIRAGE phenotypes and underwent HCT after reduced-intensity conditioning. Comorbidities, including enteropathy, electrolyte imbalances, adrenal crises, bacteremia, and lung disease, significantly led to a complicated transplant course and ultimately death in both cases. Although transplant details in 6 other cases are limited, 1 patient without the MIRAGE phenotype died of unknown cause, and 5 were surviving following HCT [7,10]. There were 4 cases of MIRAGE syndrome in our series. Before transplant, 3 of 4 cases had chronic diarrhea, malnutrition, and adrenal insufficiency. Post-HCT, we observed severe gastrointestinal fluid losses, electrolyte imbalance, and acute dehydration in these 3 cases. Whether such dramatic stool losses without an infectious etiology were secretory and whether autonomic instability could have contributed are unknown. Patients also experienced temperature and blood pressure instability, respiratory distress, and acute renal dysfunction.

Several of these medical issues are similar to those reported in the report by Sarthy et al. [14]. Despite a complicated acute transplant course, all 4 patients with MIRAGE syndrome in our series survived.

We observed a high rate of ongoing medical issues in MIRAGE syndrome transplant survivors. These include adrenocortical insufficiency, diarrhea, need for supplemental nutrition, and developmental delays. Patients with pre-existing lung disease and nephropathy continue to have these issues following HCT. Most of these issues are related to pre-existing MIRAGE syndrome manifestations. The transplant survivor reported by Wilson et al. [21] had ongoing medical issues of adrenocortical insufficiency, growth and developmental delays, and chronic lung and chronic kidney diseases.

In this series, all 6 *SAMD9L* patients had cytopenias and MDS with chromosome 7 abnormalities. We did not observe ataxia, incoordination, or other neurologic manifestations before or following transplant. On review of the literature, we found 11 additional cases of patients with *SAMD9L* mutations who had undergone HCT [5,7,11]. Although transplant details are limited, 2 patients died of complications (cerebral

Table 2
Pathogenicity Assessment of Observed *SAMD9* and *SAMD9L* Variants

Patient No.	1	2	3	4	5
Gene and Variant	*SAMD9*. Heterozygous c.2471G>A (p.Arg824Gln)	*SAMD9*. Heterozygous c.4690G>A (p.G1564S)	*SAMD9*. Heterozygous c.3406G>C (p.E1136Q)	*SAMD9*. Heterozygous c.3406G>C (p.E1136Q)	*SAMD9*. Heterozygous c.2407 G>C (p.E803Q)
Method of genetic diagnosis	WES confirmed by Sanger sequencing	WES confirmed by Sanger sequencing	WES and WGS, targeted Sanger sequencing of parent	WES and WGS, targeted Sanger sequencing of parent	WES confirmed by Sanger sequencing
SAMD9 / *SAMD9L* variant: *De novo* status	*De novo* (parentage confirmed)	Since parents negative, this should be *de novo*, but parentage not confirmed	Not *de novo*	Not *de novo*	*De novo* (parentage confirmed)
Germline source	Kidney	–	Sorted lymphocytes	Sorted lymphocytes	–
Family tested for the same variant	Sibling donor was not tested prior to BMT since the *SAMD9* variant was discovered in the recipient afterwards. Parents subsequently tested and were negative.	Parents negative	Patient no. 3 and 4 in this report, a younger sibling and their mother positive. The younger sibling had transient thrombocytopenia at birth requiring platelet transfusion.	Patient no. 3 and 4 in this report, a younger sibling and their mother positive. The younger sibling had transient thrombocytopenia at birth requiring platelet transfusion.	Parents negative
ACMG* classification	Pathogenic	Likely Pathogenic	VUS (Potentially Pathogenic†)	VUS (Potentially Pathogenic†)	Likely Pathogenic
How pathogenicity was ascribed	PS2 – *de novo*, parentage confirmed PS3 – functional study supports damaging effect PM2 – absent from controls	PM2 – absent from controls PM6 – assumed *de novo* PP3 – *in silico* prediction: deleterious PP4 – UPD7 together with MIRAGE features	PS3 – functional study supports damaging effect PM2 – absent from controls BS4 – lack of segregation in family members	PS3 – functional study supports damaging effect PM2 – absent from controls BS4 – lack of segregation in family members	PS2 – *de novo*, parentage confirmed PM2 – absent from controls
References	Jeffries et al. [18]	Not found via literature search	Schwartz et al, [8] (Leukemia), Schwartz et al, [13] (Nat Comm)	Schwartz et al, [8] (Leukemia), Schwartz et al, [13] (Nat Comm)	Not found via literature search
Patient No.	6	7	8	9	10
Gene and Variant	*SAMD9*. Heterozygous c.2318T>C (p.**I**773T)	*SAMD9L*. Heterozygous c.1877C>T; p.S626L	*SAMD9L*. Heterozygous c.1877C>T (p.S626L)	*SAMD9L*. Heterozygous c.3538T>C (p.W1180R)	*SAMD9L*. Heterozygous c.4651 G>C (p.V1551L)
Method of genetic diagnosis	WES confirmed by Sanger sequencing	WES confirmed by Sanger sequencing	WES confirmed by Sanger sequencing	WES confirmed by Sanger sequencing	WES confirmed by Sanger sequencing
SAMD9 / *SAMD9L* variant: *De novo* status	*De novo* status not known since dad not tested	Parents not tested. Aunt has the same variant. *De novo* status not known.	Parents not tested. Aunt has the same variant. *De novo* status not known.	Parents not tested. *De novo* status not known.	*De novo* (parentage confirmed)
Germline source	–	–	–	–	Skin fibroblasts
Family tested for the same variant	Mother tested and was negative. Dad unavailable for testing	Patients no. 7 and 8 in this report are nephews. A maternal aunt with cytopenias and confirmed *SAMD9L* mutation (parents not tested for *SAMD9*/*SAMD9L*), brother with cytopenias (not tested).	Patients no. 7 and 8 in this report are nephews. A maternal aunt with cytopenias and confirmed *SAMD9L* mutation (parents not tested for *SAMD9*/*SAMD9L*), brother with cytopenias (not tested).	Parents not tested	Parents negative
ACMG* classification	VUS	Likely Pathogenic	Likely Pathogenic	Likely Pathogenic	Pathogenic
How pathogenicity was ascribed	PM2 - absent from controls PP4 – subclonal 7q deletion together with MIRAGE features	PS3 – functional study supports damaging effect PM2 – absent from controls PP1 – segregates with affected family members	PS3 – functional study supports damaging effect PM2 – absent from controls PP1 – segregates with affected family members	PS3 – functional study supports damaging effect PM2 – absent from controls	PS2 – de novo PS3 – functional study supports damaging effect PM2 – absent from controls
References	Perisa et al, [17]	Schwartz et al, [8] (Nat Comm)	Schwartz et al, [8] (Nat Comm)	Schwartz et al, [8] (Nat Comm)	Ortolano et al, [19]

(*continued*)

Table 2 (*Continued*)

Patient No.	11	12			
Gene and Variant	*SAMD9L*. Heterozygous c.2957G>A (p.R986H)	*SAMD9L*. Heterozygous c.2957G>A (p.R986H)			
Method of genetic diagnosis	Sanger sequencing of peripheral blood. Confirmed by Sanger sequencing of hair follicles	Targeted NGS. Confirmed by Sanger sequencing of hair follicles			
SAMD9 / *SAMD9L* variant: *De novo* status	Not *de novo*	Not *de novo*			
Germline source	Hair follicles	Hair follicles			
Family tested for the same variant	Patients no. 11 and 12 in this report are siblings. Father positive. Mother negative.	Patients no. 11 and 12 in this report are siblings. Father positive. Mother negative.			
ACMG* classification	Likely Pathogenic	Likely Pathogenic			
How pathogenicity was ascribed	PS3 – functional study supports damaging effect PM5 – another variant (p.R986C) at the same position is pathogenic	PS3 – functional study supports damaging effect PM5 – another variant (p.R986C) at the same position is pathogenic			
References	Tesi et al, [5]; Bluteau et al, [7]; Wong et al, [12]	Tesi et al, [5]; Bluteau et al, [7]; Wong et al, [12]			

Abbreviations: WES indicates whole exome sequencing; WGS, whole genome sequencing; BMT, blood and marrow transplantation; ACMG, American College of Medical Genetics, and VUS, variant of unknown significance; NGS, Next generation sequencing.

* Each pathogenic criterion was weighted as very strong (PVS1), strong (PS1–4); moderate (PM1–6) or supporting (PP1–5) and each benign criterion was weighted as stand-alone (BA1), strong (BS1–4) or supporting (BP1–6). From Richards et al, [20].

† The *SAMD9* variant c.3406G>C (p.E1136Q) was classified as a VUS using strict ACMG criteria. We believe this variant is pathogenic based on well-established functional data from two separate experimental studies showing that it has a deleterious effect on cells. The younger sibling of the patients above also carries the variant and had transient neonatal thrombocytopenia requiring transfusion. However, the mother of these patients carries the variant as well and presently lacks an apparent phenotype. Whether she was transiently affected in the past is unknown, but this is possible as somatic revertant mosaicism is a known associated phenomenon with *SAMD9/SAMD9L* variants. Other potential mechanisms that could account for the lack of phenotypic segregation include monoallelic gene expression, incomplete penetrance, or variable expressivity. We feel this is important to note for clinical reasons in case this variant is observed in another patient.

Table 3
Transplant Characteristics and Outcomes

Patient No.	1	2	3	4	5
Gene involved	*SAMD9* (MIRAGE syndrome)	*SAMD9* (MIRAGE syndrome)	*SAMD9*	*SAMD9*	*SAMD9* (MIRAGE syndrome)
Age at HCT, years	6.7	1.4	3.3	5	1.2
Interval from diagnosis to HCT, years	6.5	0.4	0.2	0.2	0.4
Indication for HCT	Presumed congenital amegakaryocytic thrombocytopenia	MDS	MDS	MDS	MDS
Significant pretransplant issues	Secretory diarrhea, adrenocortical insufficiency, lung disease, CKD, failure to thrive	Esophageal achalasia, gastroesophageal reflux, diarrhea, failure to thrive	–	–	Diarrhea. Failure to thrive.
Donor type	HLA-identical sibling, female, bone marrow	Unrelated, 10/10 allele match, male, bone marrow	Unrelated, 8/8 allele match, female, bone marrow	Unrelated, 8/8 allele match, male, bone marrow	Father, 5/10 allele match, bone marrow
Conditioning regimen; GVHD prophylaxis	Flu/Cy/ATG; Tac/MMF	Bu/Flu/ATG; Tac/Mtx	Bu/Cy/ATG; CsA/Mtx	Bu/Cy/ATG; CsA/Mtx	Bu/Flu; posttransplant Cy, Tac/MMF
Conditioning intensity (MA / RIC)	RIC	MA	MA	MA	MA
Neutrophil engraftment, days+	13	12	16	19	14
Platelet engraftment, days+	16	30	14	15	40
Posttransplant course	Temperature & blood pressure instability, electrolyte imbalance, dehydration, hypoxia	TMA, recurrent pericardial effusions, hypoxia	VOD of liver	Pericardial effusion	TMA, pericardial effusion, VOD of liver
Intensive care	Severe hypertension	No	Respiratory distress, did not require intubation	Respiratory distress, required intubation	Respiratory failure, did not require intubation
Acute GVHD / Chronic GVHD	No / No	No / Yes	No / No	No / No	No / No
Chimerism	99% donor	100% donor	100% donor	99% donor	100% donor
Post-HCT hematologic outcome	Normal blood counts, no monosomy 7	Normal blood counts, no monosomy 7, resolution of MDS	Resolution of MDS, no chr. 7 finding	Resolution of MDS, no chr. 7 findings	Normal blood counts, no monosomy 7, resolution of MDS
Survival status	Alive; 2.4 y post-HCT	Alive; 3.8 y post-HCT	Alive; 3.2 y post-HCT	Alive; 3 y post-HCT	Alive; 1.4 y post-HCT
Current health status	Secretory diarrhea, enteral feeds, low weight and height, thriving, developmental delay, CKD, hypertension, adrenal insufficiency	Recurrent aspiration pneumonias, chronic lung disease, malnutrition, diarrhea, developmental delay, thriving, adrenal insufficiency	School performance issues	Learning disabilities	Supplemental feeds, hypoglycemia episodes, diarrhea, low weight and height, thriving, developmentally delay

(*continued*)

Table 3 (*Continued*)

Patient No.	6	7	8	9	10
Gene involved	*SAMD9* (MIRAGE syndrome)	*SAMD9L*	*SAMD9L*	*SAMD9L*	*SAMD9L*
Age at HCT, years	7.8	12.8	2.3	8.3	2
Interval from diagnosis to HCT, years	5.5	0.2	1.4	0.2	1.3
Indication for HCT	MDS	Presumed dyskeratosis congenita	MDS	MDS	MDS
Significant pretransplant issues	Hypertension, chronic kidney disease, asthma	Obesity (BMI 34, >97th percentile for age)	Obesity (BMI 27, >97th percentile for age)	–	HLH therapy. E. coli sepsis, pancolitis, ecthyma gangrenosum, aspergillus and candida sepsis
Donor type	Unrelated, 10/10 allele match, male, bone marrow	Unrelated double cord blood, male (5/6 allele match), female (5/6 allele match)	Unrelated cord blood, 6/6 allele match, female	HLA-identical sibling, female, bone marrow	Unrelated, 9/10 allele match, bone marrow
Conditioning regimen; GVHD prophylaxis	Flu/Mel/Alemtuzumab; Tac/MMF	Flu/Mel/Alemtuzumab; Tac/MMF	Flu/Cy/TBI; CsA/MMF	Cy/TBI/Ara-C	Bu/Cy/ATG
Conditioning intensity (MA / RIC)	RIC	RIC	MA	MA	MA
Neutrophil engraftment, days+	19	No	13	17	18
Platelet engraftment, days+	19	No	12	31	No
Posttransplant course	Blood pressure instability, electrolyte imbalance, fevers, hypoxia	Restrictive lung disease	Parainfluenza with respiratory failure, renal dysfunction	Culture negative sepsis, bleeding gastric ulcer, hemorrhagic cystitis	Coronavirus respiratory tract infection, VOD of liver with respiratory failure, defibrotide, diffuse alveolar hemorrhage
Intensive care	No	No	Respiratory failure	Systemic inflammatory response syndrome	Respiratory failure, required intubation
Acute GVHD / Chronic GVHD	No / No	No / No	Yes (Grade II, GI, resolved)/ No	No / No	Not evaluable / Not evaluable
Chimerism	98% donor	0% donor	99% donor	100% donor	Not done
Post-HCT hematologic outcome	Normal blood counts	Graft failure	Resolution of MDS, no chr. 7 finding	Resolution of MDS, no chr. 7 finding	Neutrophil engraftment. Bone marrow not assessed
Survival status	Alive; 4.1 y post-HCT	Died of refractory AML; 1.1 y post-HCT	Alive; 2.3 y post-HCT	Alive; 14.7 y post-HCT	Died at day +23 post-HCT from complications related to VOD of liver
Current health status	Adrenal insufficiency, diarrhea, hypotension, CKD, urethrocutaneous fistula, developmental delay, thriving	N.A.	CKD	Doing well	N.A.

(*continued*)

Table 3 (*Continued*)

Patient No.	11	12			
Gene involved	*SAMD9L*	*SAMD9L*			
Age at HCT, years	2.1	1.8			
Interval from diagnosis to HCT, years	0.5	0.5			
Indication for HCT	MDS	MDS			
Significant pretransplant issues	Otitis media, croup, roseola	Alpha hemolytic streptococcal sepsis			
Donor type	Unrelated, 10/10 allele match, female, bone marrow	Unrelated, 10/10 allele match, female, bone marrow			
Conditioning regimen; GVHD prophylaxis	Bu/Cy; Tac/Mtx	Bu/Cy; Tac/Mtx			
Conditioning intensity (MA / RIC)	MA	MA			
Neutrophil engraftment, days+	19	9			
Platelet engraftment, days+	17	12			
Posttransplant course	Uneventful	VOD of liver, hemolysis, coagulopathy			
Intensive care	No	VOD			
Acute GVHD / Chronic GVHD	Yes (Grade II, skin, gut, resolved) / Yes skin, mild	No / No			
Chimerism	100% donor	100% donor			
Post-HCT hematologic outcome	Normal blood counts, no monosomy 7, resolution of MDS	Normal blood counts, no monosomy 7, resolution of MDS			
Survival status	Alive; 5.3 y post-HCT	Alive; 1.3 y post-HCT			
Current health status	Doing well	Doing well			

Abbreviations: ATG (anti-thymocyte globulin); Ara-C (cytosine arabinoside); BU (busulfan); BMI (body mass index); Chr. 7 (chromosome 7); CKD (chronic kidney disease); Cy (cyclophosphamide); CsA (cyclosporine A); GI (gastrointestinal); Flu (fludarabine); HLH (hemophagocytic lymphohistiocytosis); MA (myeloablative); MDS (myelodysplastic syndrome); Mel (melphalan); MIRAGE syndrome (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy); MMF (mycophenolate mofetil); Mtx (methotrexate); N.E. (not evaluable); RIC (reduced intensity conditioning); Tac (tacrolimus); TBI (total body irradiation); TMA (thrombotic microangiopathy); and VOD (veno-occlusive disease)

Table 4
Summary of Available Clinical Data on Immune Reconstitution

	Patient No.								
Characteristic	1	2	5	6	3	4	8	11	12
Gene mutation	*SAMD9*	*SAMD9*	*SAMD9*	*SAMD9*	*SAMD9*	*SAMD9*	*SAMD9L*	*SAMD9L*	*SAMD9L*
MIRAGE phenotype	Yes	Yes	Yes	Yes	No	No	No	No	No
Lymphocyte enumeration									
1 month post-HCT									
ALC per cumm	570	678	470	252	924	546	288	NA	1512
2 months post-HCT									
ALC per cumm	1970	1000	1320	864	2368	240	826	NA	112
3 months post-HCT									
ALC per cumm	2080	1307	1650	ND	ND	1125	410	NA	370
CD3 per cumm	375	891	ND	ND	ND	ND	ND	NA	NA
CD4 per cumm	250	369	ND	ND	ND	ND	ND	NA	NA
CD8 per cumm	83	486	ND	ND	ND	ND	ND	NA	NA
NK cells per cumm	520	167	ND	ND	ND	ND	ND	NA	NA
CD19 per cumm	1145	249	ND	ND	ND	ND	ND	NA	NA
Serum IgG, mg/dL	123	1120	519	822	ND	395	ND	NA	NA
6 months post-HCT									
ALC per cumm	2500	840	4630	1254	ND	544	935	980	981
CD3 per cumm	1150	726	2224	390	ND	ND	ND	ND	451
CD4 per cumm	600	308	1308	277	ND	ND	ND	ND	216
CD8 per cumm	500	377	828	86	ND	ND	ND	ND	212
NK cells per cumm	900	114	916	193	ND	ND	ND	ND	193
CD19 per cumm	450	0	1264	662	ND	ND	ND	ND	337
Serum IgG, mg/dL	346	254	915	752	522	218	ND	389	521
12 months post-HCT									
ALC per cumm	6100	1801	8200	1938	ND	770	2220	1490	4070
CD3 per cumm	3841	999	6232	1212	ND	ND	ND	1356	2426
CD4 per cumm	1829	495	3526	737	ND	ND	ND	374	1548
CD8 per cumm	1890	459	2460	362	ND	ND	ND	884	829
NK cells per cumm	549	185	656	178	ND	ND	ND	97	422
CD19 per cumm	1646	617	1148	502	ND	ND	ND	127	1121
Serum IgG, mg/dL	759	623		371	ND	300	841	351	NA

Patient 1 (*SAMD9* with MIRAGE): Protein-losing enteropathy. Intravenous immunoglobulin (IVIG) infusions. Patient 2 (*SAMD9* with MIRAGE): Chronic diarrhea. Patient 3 (*SAMD9* without MIRAGE): Lymphocyte enumeration 3 years post-HCT, ALC 4555, CD3 3160, CD4 1330, CD8 1610, NK cells 480, CD19 740, all in per cumm. Patient 4 (*SAMD9* without MIRAGE): Lymphocyte enumeration 3 years post-HCT, ALC 3700, CD3 2530, CD4 1090, CD8 1140, NK cells 400, CD19 770, all in per cumm. Patient 5 (*SAMD9* with MIRAGE): IVIG infusions monthly until 1 year post-HCT. Patient 6 (*SAMD9* with MIRAGE): IVIG infusions monthly until 6 months post-HCT. Patient 7 (*SAMD9L*): Not included in the table. ALC 286 on day +60. Graft failure. Patient 8 (*SAMD9L*): Lymphocyte enumeration 5 years post-HCT, ALC 3600, CD3 2630, CD4 1200, CD8 1310, NK cells 120, CD19 810, all in per cumm. Patient 9 (*SAMD9L*): Not included in the table. Underwent HCT 14 years ago. Data not available. Patient 10 (*SAMD9L*): No data. The patient died of transplant complications on day +23. Patient 11 (*SAMD9L*): Intermittent IVIG infusions. Patient 12 (*SAMD9L*): Intermittent IVIG infusions.
ALC indicates absolute lymphocyte count; ND, not done; NK, natural killer; IgG, immunoglobulin G.

hemorrhage and infection, 1 each), 1 had unknown survival status, and 8 were alive. Of the surviving patients, 1 had pulmonary fibrosis, and 3 had neurologic issues.

Mutations in *SAMD9* and *SAMD9L* add to a growing list of recently described heritable conditions associated with cytopenias, marrow failure, MDS, and AML [1,7,8]. Although these patients can be managed symptomatically with transfusions and treatment of infections, the only curative treatment is with allogeneic HCT.

Indications and timing of HCT in these patients are not straightforward because marrow cells can undergo somatic genetic correction events and spontaneous blood count recovery [4,8,12,22]. In our series, there was an interval of several years from initial presentation to development of bone marrow failure or MDS in 2 cases. Most patients in our series underwent transplantation for MDS with transfusion dependence, and a diagnosis of *SAMD9/SAMD9L* was made retrospectively from archived specimens. Affected siblings of patients who underwent transplantation have been followed without transplant; however, these are anecdotal case reports, and long-term data are needed [8,11].

Patients who have relatively stable blood counts and do not show signs of development of MDS or AML may continue to be closely observed. However, in our view, patients who develop significant marrow failure (including if clinically symptomatic with infections, anemia, bleeding, and/or transfusion dependence), meet morphologic criteria of MDS, develop monosomy 7 or 7q-, or develop other cytogenetic abnormalities associated with myeloid malignancies should be evaluated for allogeneic HCT. Any potential family donors must undergo genetic evaluation for *SAMD9/SAMD9L* mutation as well.

In conclusion, in this small series of patients, we found that most patients with *SAMD9/SAMD9L* mutations tolerated transplant conditioning, with a high rate of engraftment and resolution of MDS or marrow failure. Clinically significant comorbidities were common in MIRAGE syndrome cases and contributed to unique adverse events in the acute post-transplant phase. These patients continue to require ongoing management and multispecialty care for syndrome-related nonhematologic manifestations.

More data are needed to define timing of HCT in *SAMD9/SAMD9L* patients and further refine conditioning regimens as well as management of patients with significant syndrome-related comorbidities. National and international transplant registries should be queried to examine reported outcomes in larger patient cohorts. Finally, long-term follow-up and care guidelines are needed for the survivors.

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