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Spontaneous remission and loss of monosomy 7: a window of opportunity for young children with SAMD9L syndrome

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Authorship Contributions

M.E. and F.A. designed the research, analyzed, interpreted clinical data, and wrote the manuscript. C.M., C.M.N., and M.W.W. contributed to the manuscript conception. M.E., B.S., A.Y., C.M.N., M.S., J.S., B.d.W., J.v.d.W.T.B., M.D., M.G.S., S.P., R.B., C.K., M.N., and M.C.F. were involved in patient care, testing, and data presentation. All authors contributed to the manuscript and approved its final version.

Disclosure of Conflicts of Interest

The authors disclose no conflicts of interest.

Data-sharing statement

All data relevant to the study are included in the article or uploaded as supplementary information.

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Abstract

Monosomy 7 is the most common cytogenetic abnormality in pediatric myelodysplastic syndrome (MDS) and associated with a high risk of disease progression. However, in young children, spontaneous loss of monosomy 7 with concomitant hematologic recovery has been described, especially in the presence of germline mutations in *SAMD9* and *SAMD9L* genes. Here, we report on our experience of close surveillance instead of upfront hematopoietic stem cell transplantation (HSCT) in seven patients diagnosed with *SAMD9L* syndrome and monosomy 7 at a median age of 0.6 years (0.4 - 2.9). Within 14 months from diagnosis, three children experienced spontaneous hematological remission accompanied by a decrease in monosomy 7 clone size. Subclones with somatic *SAMD9L* mutations in *cis* were identified in five patients, three of whom attained hematological remission. Two patients acquired *RUNX1* and *EZH2* mutations during the observation period, of whom one progressed to MDS with excess of blasts (MDS-EB). Four patients underwent allogeneic HSCT at a median time of 26 months (14 - 40) from diagnosis for MDS-EB, necrotizing granulomatous lymphadenitis, persistent monosomy 7, and severe neutropenia. At last follow-up, six patients were alive, while one passed away due to transplant-related causes. These data confirm previous observations that monosomy 7 can be transient in young children with *SAMD9L* syndrome. However, they also indicate that delaying HSCT poses a substantial risk of severe infection and disease progression. Finally, surveillance of patients with *SAMD9L* syndrome and monosomy 7 is critical to define the evolving genetic landscape and to determine the appropriate timing of HSCT.

Introduction

Loss of chromosome 7 and partial deletion of its long arm (i.e., monosomy 7 and del(7q)) are frequent non-random cytogenetic aberrations in pediatric patients with myeloid malignancies, including myelodysplastic syndrome (MDS).¹⁻³ Several tumor suppressors and regulators of myeloid differentiation have been identified on chromosome 7. Among them, deletion of *EZH2*, *MLL3/KMT2C*, *SAMD9L*, and *CUX1* has been shown to promote malignant transformation in mouse models.⁴⁻⁸ The presence of monosomy 7 is generally associated with a high risk of disease progression and acquisition of oncogenic mutations, and timely allogeneic hematopoietic stem cell transplantation (HSCT) is warranted.^{1,9-11} However, Scheurlen et al. first reported in 1994 on a 14-month-old boy with MDS and monosomy 7 that achieved spontaneous hematologic recovery within two years.¹² Subsequently, further case reports and series of transient monosomy 7 in infants and young children with MDS have been published demonstrating spontaneous hematologic recovery upon loss of monosomy 7.¹³⁻¹⁷

Chromosome 7 aberrations have been associated with several germline conditions predisposing to hematopoietic neoplasia, such as GATA2 deficiency syndrome and Fanconi anemia.¹⁸⁻²⁰ In GATA2 deficiency syndrome, 40% of pediatric patients with cytopenia carry monosomy 7, del(7q), or an unbalanced translocation der(1;7), with the highest prevalence in adolescence.^{18,19} Recently, a growing body of research has highlighted the association of monosomy 7 and del(7q) with germline mutations in *sterile alpha-motif domain-containing protein 9* (*SAMD9*) and *SAMD9-like* (*SAMD9L*).^{19,21,22}

Gain of function (GOF) mutations in *SAMD9* and its paralogue *SAMD9L* were first reported in 2016 in children with MIRAGE (myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotype, enteropathy) syndrome and ataxia pancytopenia syndrome (ATXPC), respectively.^{19,23,24} Our recent analysis of pediatric patients with the histopathological phenotype of refractory cytopenia of childhood (RCC) and MDS enrolled in the registry of the European Working Group of MDS in Childhood (EWOG-MDS) revealed that patients with germline *SAMD9* and *SAMD9L* mutations presented with a broad and overlapping phenotypic spectrum. The majority of cases presented with RCC, whereas MDS with excess of blasts (MDS-EB) was diagnosed in only 7% of patients. Furthermore, chromosome 7 aberrations were observed in 55% of all *SAMD9*- and *SAMD9L*-mutated cases.¹⁹

SAMD9 and *SAMD9L* are located adjacent to each other on chromosome 7. Germline *SAMD9/9L* GOF mutations result in reduced proliferation and survival of hematopoietic stem and progenitor cells (HSPCs).^{15,19,25,26} Acquisition of monosomy 7 and del(7q) can be considered somatic rescue events because they result in a non-random loss of the mutant allele, conferring an increased competitive fitness of HSPCs. Yet, concomitant loss of tumor suppressors located on chromosome 7 renders monosomy 7 and del(7q) a maladaptive somatic mechanism with potential for leukemic transformation. Other somatic rescue events frequently detected in bone marrow (BM) cells of affected patients include somatic loss of function (LOF) mutations of *SAMD9/9L* in *cis* and uniparental isodisomy 7q (UPD7q) with loss of the mutated allele.^{19,21,25,27}

Due to the strong selection pressure, multiple hematopoietic clones with clone-defining somatic mutations can simultaneously exist within the marrow. Moreover, in line with the distinct plasticity of hematopoiesis in young children, the polyclonal composition of BM cells may change over

time.¹⁹ Consequently, monosomy 7 may spontaneously disappear in patients with *SAMD9L* germline disorders, followed by hematologic recovery.^{15,16,25,27} This phenomenon, primarily observed in infants, represents a form of “natural gene therapy” that might spare patients from therapy like HSCT. However, little is known about the frequency, natural history of loss of monosomy 7, and factors influencing outcome of affected patients.

Methods

Seven patients less than five years of age diagnosed with a *SAMD9L* germline disorder with monosomy 7 and normal blast percentage in BM were followed closely to allow for spontaneous recovery. BM examinations every three to four months included histopathology, cytogenetics, and *SAMD9L* sequencing. Six of the seven patients were enrolled in the prospective study EWOG-MDS 2006 (www.clinicaltrials.gov; #NCT00662090); for patient 5 (P5), parental consent was obtained for the respective data analyses. University of Freiburg institutional ethics committee approved the research (ethics vote no. 247/05). Patient 2 (P2, D1300) has already been described by Sahoo et al.¹⁹ The diagnosis of RCC and MDS-EB was established according to the International Consensus Classification (ICC) of hematologic neoplasms.²⁸ Cytogenetics included classical karyotyping and interphase fluorescence-in-situ-hybridization (FISH). Targeted Next-Generation Sequencing (NGS) of *SAMD9L* allowed determination of the variant allele frequency (VAF) and detection of newly acquired somatic *SAMD9L* mutations in *cis*. To screen for somatic oncogenic mutations and corrective UPD7q, a custom-made NGS panel including genes frequently mutated in myeloid neoplasia (in the following called “myeloid NGS panel”, see supplemental information 1) and single nucleotide polymorphism (SNP) array were performed; data from the BM sample prior to HSCT or at last follow-up is reported. The American College of Medical Genetics and Association for Molecular Pathology (ACMG-AMP) 2015 guidelines were employed for the interpretation of variants identified in the myeloid NGS panel.²⁹

Results

Clinical presentation with pancytopenia, immunodeficiency, and multiple non-hematological phenotypes

We report on seven patients with *SAMD9L* germline disorder who presented with cytopenia and monosomy 7 at a median age of 0.6 years (range 0.4 – 2.9 years). At diagnosis, all patients had moderate to severe neutropenia and three children (P1, P2, P3) required platelet transfusions. Six patients had normocytic (n=5) or macrocytic (n=1) anemia, one patient had a normal hemoglobin concentration with macrocytosis of red cells (P5). Histopathology was compatible with RCC in all seven patients. Immunodeficiency with a variable clinical presentation was present in six patients. The most consistent findings of immunological impairment were hypogammaglobulinemia found in five patients (P1, P2, P5, P6, P7) and B and NK cell deficiency present in four (P2, P5, P6, P7). Patient characteristics are summarized in Table 1 and in the supplement; complete genetic data are listed in Table S1.

Six of the seven patients had a non-hematological phenotype compatible with *SAMD9L* germline disorder (Table 1). Three patients were born small for gestational age (P1, P2, P7). Patient 1 (P1) was also diagnosed with cerebellar atrophy and global developmental delay. Patient 2 (P2) was a hypotrophic preterm neonate born at 36 gestational weeks with bilateral cleft lip and

palate. Patient 3 (P3) presented with macrocephaly. Patient 5 (P5) was a triplet born preterm at 30 gestational weeks who developed hydrocephalus requiring a ventriculoperitoneal shunt in the first year of life. Patient 6 (P6) presented with mild macrocephaly and short thumbs. Patient 7 (P7) had enlarged eye bulbs and failure to thrive.

Spontaneous hematopoietic recovery and clinical course

With a median follow-up of 43 months (range 40 – 55), three of the seven patients (P3, P4, P6) experienced spontaneous regression of the monosomy 7 (Figure 1, Figure S1). In patient 4 (P4) and patient 6 (P6), monosomy 7 was no longer detectable six and 16 months from diagnosis, respectively. P4 presented at 7 months of age with pancytopenia, requirement for platelet transfusions, and a normal karyotype. Three months after the initial presentation, a small monosomy 7 clone of 7% was noted, which was no longer detectable three months later. Concurrently, the absolute neutrophil count (ANC) slowly increased to $1.23 \times 10^9/L$ at three months and $3.97 \times 10^9/L$ at six months from diagnosis, and hemoglobin concentration and platelet count reached normal values at six and 14 months, respectively. Concomitantly, BM cellularity increased to an age-adjusted normal value. P6 presented with neutropenia, macrocytosis, and moderate thrombocytopenia at the age of 2.8 years. BM analysis was compatible with hypocellular RCC with a monosomy 7 clone in 63% of BM interphases. The ANC had slowly increased seven months after diagnosis and reached close to normal values (ANC $1.47 \times 10^9/L$) 21 months from diagnosis. By that time, the monosomy 7 clone was no longer detectable and hemoglobin and platelet count were normalized at 16 and 32 months from diagnosis, respectively. P3 presented with pancytopenia at the age of four months. BM histopathology was compatible with hypocellular RCC with monosomy 7 in 37% of BM interphases. Nine months later, hemoglobin concentration and platelet count had normalized, but isolated neutropenia (ANC $0.38 \times 10^9/L$) persisted. Thirteen months after diagnosis, trephine biopsy showed a normocellular BM according to age and the complete blood count (CBC) gradually improved. Further invasive procedures were denied, the child was alive with a normalized CBC 4.3 years after diagnosis (age 4.5 years).

All four patients that did not experience spontaneous remission (P1, P2, P5, P7) received an allogeneic HSCT with a median interval from diagnosis to HSCT of 26 months (range 14-40) (Figure S1). Indications for HSCT were severe neutropenia and/or bacterial infection (P1, P7), persistence of monosomy 7 (P5), and disease progression to MDS-EB (P2). Three of the four transplanted patients (P1, P5, P7) were alive with stable engraftment and complete donor chimerism at last follow-up. Patient 2 (P2) who succumbed to transplant-related causes had disease progression prior to HSCT. At the age of 15 months (i.e., 10 months after diagnosis), the patient developed severe neutropenia prompting a 12-day treatment course with G-CSF. Subsequently, the BM blast percentage and cellularity increased consistent with progression to MDS-EB and persisted after G-CSF withdrawal. At the time, two *RUNX1* variants (see below) were detected in the BM. After a myeloablative conditioning regimen and an allogeneic HSCT from a matched unrelated donor, the patient developed acute respiratory distress syndrome and veno-occlusive disease and died in hematologic remission.

Somatic events influencing disease outcome

The three patients with spontaneous remission (P3, P4, P6) had somatic *SAMD9L* LOF mutations in *cis* known to disrupt the germline allele. Figure 1 depicts the courses of the VAF of *SAMD9L*, monosomy 7, and ANC. P3 acquired a somatic *SAMD9L* variant (*SAMD9L* c.1765C>T, p.R589*) 13 months from diagnosis (VAF 12%), molecular analyses at later time points could not be obtained. P4 acquired two somatic *SAMD9L* variants with a VAF of 27% (*SAMD9L* c.683G>A, p.C228Y) and 4% (*SAMD9L* c.2699>G, p.Y900C) six months from diagnosis concomitantly with loss of the previously diagnosed monosomy 7. Both *SAMD9L* clones remained stable over time, a third somatic *SAMD9L* variant (*SAMD9L* c.3562C>T, p.R1188*, VAF 4%) was detected 41 months after diagnosis. P6 harbored a somatic *SAMD9L* variant (*SAMD9L* c.4224dupA, p.Q1409Tfs*49) with a VAF of 6% at diagnosis. The somatic *SAMD9L* variant slowly increased to 35%, while the monosomy 7 clone decreased in size over time and subsequently disappeared at 16 months from diagnosis.

Importantly, we also detected somatic *SAMD9L* mutations in *cis* in two patients (P2, P7) who did not experience hematological recovery and/or a decrease in monosomy 7 clone size (Table S1, Figure S1). P2 acquired a somatic *SAMD9L* variant one month after diagnosis (*SAMD9L* c.768dup, p.K257*, VAF 6%). Within twelve months, the VAF of this variant increased to 23%, however, the disease progressed to MDS-EB. P7 had a large monosomy 7 clone at diagnosis and acquired a somatic *SAMD9L* variant (*SAMD9L* c.2385C>A, p.Y795*) with a VAF of 6% six months later. The patient received an allograft for persistent severe neutropenia ten months later.

The myeloid NGS panel analysis demonstrated that two patients (P2, P5) had acquired oncogenic mutations during the course of their disease (Table S1, Figure S1). P2 acquired two pathogenic *RUNX1* mutations twelve months from diagnosis (*RUNX1* c.317G>A, p.W106*; *RUNX1* c.496_508+2dup), which was accompanied by disease progression to MDS-EB. In P5, a pathogenic variant in *RUNX1* (*RUNX1* c.593A>G, p.Asp198Gly) and a variant of unknown significance in *EZH2* (*EZH2* c.1672+3_1672+4del) were detected 30 months after diagnosis. At this time point, the monosomy 7 clone size had increased again to 12% following an initial decrease from 52% to 4% and the patient had already been scheduled for HSCT for persistent monosomy 7.

Discussion

Somatic genetic rescue events (SGRs) that (at least in part) abrogate an underlying deleterious germline defect and thereby confer a selective advantage have been described in several inherited bone marrow failure and leukemia predisposition syndromes.³⁰⁻³⁶ Prime examples are *SAMD9/9L* germline disorders with multiple SGR events typically resulting in a polyclonal bone marrow (Figure 2).¹⁹

This case series describes the natural history of transient monosomy 7 in seven children with *SAMD9L* germline disorders below the age of five years at diagnosis. Spontaneous hematologic recovery was observed in three children; complete loss of monosomy 7 was demonstrated in two children and can be assumed in the third patient who was withdrawn from follow-up BM studies but normalized his CBC. In all three patients, improvement of blood counts was accompanied by the emergence and/or expansion of clones harboring somatic *SAMD9L* mutations in *cis* as well as a gradual decrease in monosomy 7 clone size. However, somatic

SAMD9L mutations in *cis* were also detected in two patients who did not experience hematologic recovery. This finding underlines that the acquisition of adaptive SGR clones such as somatic *SAMD9L* mutations in *cis* does not reliably predict loss of monosomy 7 or favorable clinical outcome.¹⁹ Interestingly, we did not detect somatic genetic reversion by UPD7q with non-random loss of the *SAMD9L* germline variant in either of the two patients with spontaneous remission and BM material available for SNP array. Whether somatic *SAMD9L* mutations in *cis* and UPD7q are functionally equivalent with respect to long-term sustainability of normal hematopoietic function is currently unknown.

It is well established that the somatic mutational landscape in children with MDS differs from that observed in adults.^{19,21,37-39} The nature of somatic variants noted in pediatric MDS is largely dependent on the underlying genetic predisposition,^{19,34,36,40,41} the morphological subtype (MDS-EB vs. RCC), and the karyotype (monosomy 7 vs. other karyotypes).^{15,21} Our recently published data demonstrated that *SAMD9/9L* mutated patients most frequently harbored mutations in *SETBP1*, *ASXL1*, *RUNX1*, *EZH2*, and the *RAS* pathway genes, thereby resembling the somatic mutational pattern of *GATA2* deficiency and MDS with monosomy 7 without underlying *GATA2* or *SAMD9/9L* germline mutations.¹⁹ In two of the patients described here, we observed the emergence of somatic mutations in *RUNX1* and *EZH2* during BM surveillance. Somatic variants in *RUNX1* play a critical role in leukemic transformation in a number of predisposition syndromes, specifically in severe congenital neutropenia (SCN) and Fanconi anemia: Skokowa et al. detected somatic *RUNX1* mutations in 64% of pediatric patients with MDS/AML following SCN,⁴⁰ while Sebert et al. described *RUNX1* alterations in 34% of FA patients with clonal hematopoiesis.³⁶

Meticulous description of the sequence of acquisition of individual somatic aberrations during leukemogenesis is a prerequisite for unraveling their impact on outcome of patients with pediatric MDS. Schwartz et al. reported that the presence of somatic *SETBP1* mutations is associated with a poor outcome in patients with MDS-EB.²¹ *SETBP1* mutations are often noted in the context of monosomy 7.¹⁵ In our previous report, 16 of 59 patients (27%) with *SAMD9/9L* germline disorders and normal blast count carried somatic oncogenic mutations, and twelve of the 16 patients had monosomy 7. The presence of monosomy 7 had a negative impact on patient outcome; among patients with *SAMD9/9L* syndrome and normal blast count, 5-year overall survival was 93% in patients with normal karyotype and 73% in the presence of monosomy 7.¹⁹

To the best of our knowledge, somatic genetic rescue with complete hematological recovery in *SAMD9L* syndrome and monosomy 7 has only been observed in patients less than 5 years of age.^{15,16,19,22,42} This age dependency may indicate that BM plasticity substantially decreases during the first years of life. At the same time, it provides a window of opportunity for bone marrow surveillance that might spare some of these affected children from allogeneic HSCT. Reoccurrence of monosomy 7 has not been described, the longest reported follow-up is 20 years.¹⁵

Nevertheless, monosomy 7 remains a risk factor for potential disease progression. Although BM sampling is an invasive procedure requiring analgesic sedation or anesthesia in small children, frequent BM examinations (e.g., q3 - 4 month) are advised to detect emerging oncogenic mutations early during malignant transformation. Of note, two of the seven patients included in the surveillance strategy reported were transplanted for infection and severe neutropenia. When initiating a surveillance strategy, treating physicians need to be aware that these young children with severe to moderate neutropenia, B and NK cell deficiency, and/or hypogammaglobulinemia are at increased risk for life-threatening infections.

This case series has two main limitations. First, the small number of patients renders it difficult to draw definitive conclusions about patient management. Furthermore, since our myeloid NGS panel has a detection limit of 5%, small somatic clones might have been missed in some patients. Future studies of larger patient cohorts using more sensitive sequencing methods such as duplex unique molecular identifiers (UMI) sequencing⁴³ or single-cell sequencing approaches could address these open questions.

In summary, surveillance instead of upfront HSCT can give some patients less than 5 years of age with *SAMD9L* syndrome and monosomy 7 a chance to experience spontaneous hematological remission. However, stringent indications for HSCT are recommended to render this expectant approach a safe procedure in this young patient population. Although the mechanism of loss of monosomy 7 is not fully elucidated yet, clinical experience indicates that expansion of clones with somatic rescue mutations in *cis* is sufficient to allow for hematopoietic regeneration in some patients. Such “natural gene therapy” provides a promising template for novel gene therapy approaches. Introducing stop mutations, which are the most frequent somatic rescue events in *SAMD9L* disease, is technically well feasible rendering *SAMD9/9L* germline syndromes most amenable to gene therapy.

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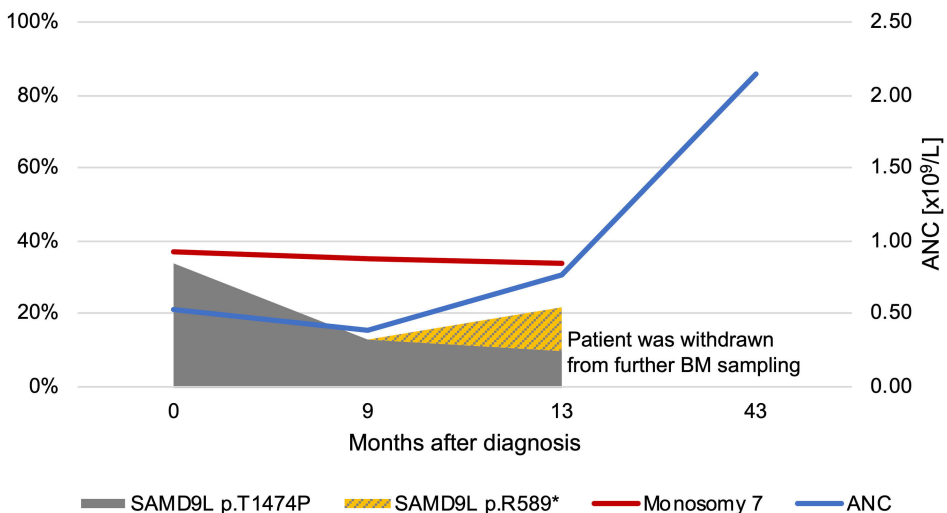
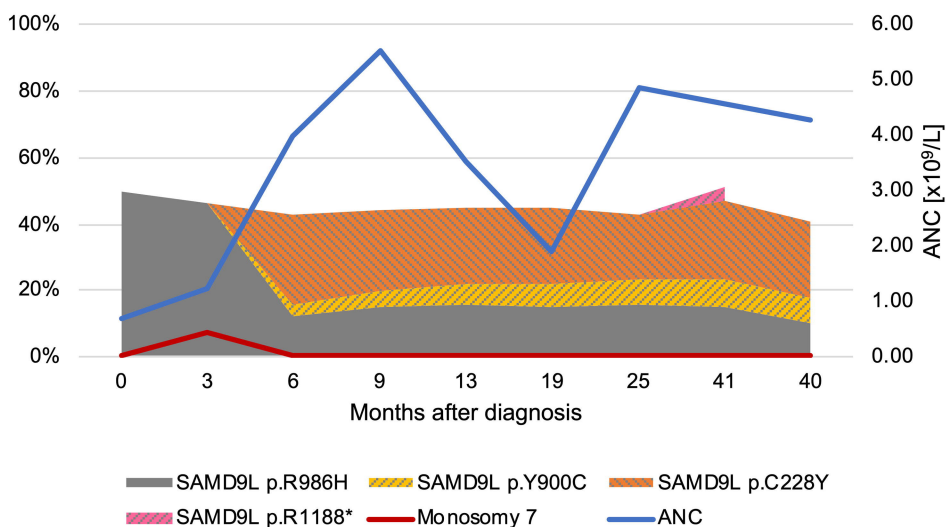
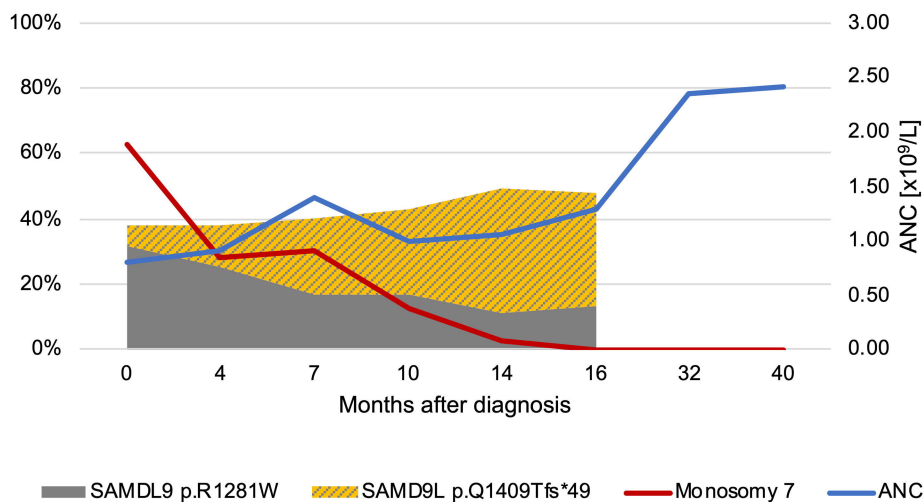
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Patient no. (UPN)	Sex	Age at dx (mo)	CBC at dx (WBC, ANC, PLT, Hb, MCV)	Non-hematological phenotype	Somatic <i>SAMD9L</i> mutation at last FU (no.)	Monosomy 7 clone size at dx	Somatic cancer gene mutation at last FU (no.)	Age at HSCT (mo)	Status at last FU
				Immunological phenotype		Loss of monosomy 7		HSCT indication	
P1 (D1297)	M	6	6.40 x 10 ⁹ /L, 0.70 x 10 ⁹ /L, 100 x 10 ⁹ /L [#] , 9.5 g/dL, 79 fL	SGA, cerebellar atrophy, global developmental delay	No	73%	No	46	Alive
				Hypogammaglobulinemia		No		Necrotizing granulomatous lymphadenitis	
P2 (D1300)	F	5	3.90 x 10 ⁹ /L, 0.39 x 10 ⁹ /L, 3 x 10 ⁹ /L, 6.7 g/dL, 75 fL	Preterm infant (36 GW), SGA, cleft lip and palate	Yes (1)	80%	<i>RUNX1</i> (2)	19	Dead
				Hypogammaglobulinemia, B/NK cell deficiency		No		MDS-EB	
P3 (GR012)	M	4	3.14 x 10 ⁹ /L, 0.35 x 10 ⁹ /L, 12 x 10 ⁹ /L, 8 g/dL, 80 fL	Macrocephaly	Yes (1)	37 %	No	N/A	Alive
				None		N/A			
P4 (B063)	M	7	7.10 x 10 ⁹ /L, 0.71 x 10 ⁹ /L, 107 x 10 ⁹ /L [#] , 8 g/dL, 78 fL	None	Yes (3)	7%	No	N/A	Alive
				None		Yes			
P5 (KM)	F	17	4.50 x 10 ⁹ /L, 0.50 x 10 ⁹ /L, 170 x 10 ⁹ /L, 12.3 g/dL, 92 fL	Preterm triplet (30 GW), hydrocephalus	No	52%	<i>RUNX1</i> (1), <i>EZH2</i> (2)	53	Alive
				Hypogammaglobulinemia, B/NK cell deficiency		No		Persistent -7	
P6 (CZ132)	F	34	4.10 x 10 ⁹ /L, 0.81 x 10 ⁹ /L, 46 x 10 ⁹ /L, 11 g/dL, 94 fL	Mild macrocephaly, short thumbs	Yes (1)	63%	No	N/A	Alive
				Hypogammaglobulinemia, B cell deficiency		Yes			
P7 (A146)	F	15	2.54 x 10 ⁹ /L, 0.25 x 10 ⁹ /L, 79 x 10 ⁹ /L, 7.7 g/dL, 89 fL	SGA, failure to thrive, big eye bulbs	Yes (1)	66 %	No	32	Alive
				Hypogammaglobulinemia, B/NK cell deficiency		No		Severe neutropenia	

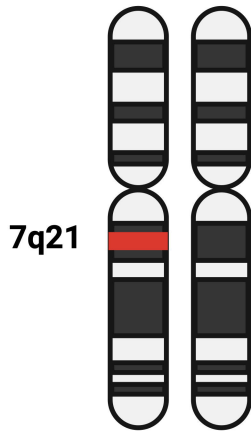
Table 1. Phenotype and clinical course of seven patients with *SAMD9L* germline mutations and monosomy 7. Abbreviations: No.: number. UPN: unique patient number. Dx: diagnosis. Mo: months. CBC: complete blood count. WBC: white blood cells, ANC: absolute neutrophil count, PLT: platelets. Hb: hemoglobin. MCV: mean corpuscular volume. FU: Follow-up. HSCT: hematopoietic stem cell transplantation. FU: follow-up. SGA: Small for gestational age. N/A: not applicable. GW: weeks of gestation. MDS-EB: MDS with excess blasts. #post-transfusion.

Figure 1. Course of *SAMD9L* VAF, monosomy 7 (analyzed by FISH), and ANC of patient 3, 4, and 6. Abbreviations: FISH: Fluorescence-in-vitro-hybridization. VAF: Variant allele frequency. ANC: absolute neutrophil count.

Figure 2. Somatic rescue events in *SAMD9/9L* germline disorders. Abbreviations: UPD: Uniparental isodisomy. LOF: loss-of-function. HSPCs: hematopoietic stem and progenitor cells. Created with BioRender.com.

Patient 3 (GR012)**Patient 4 (B063)****Patient 6 (CZ132)**

**Normal SAMD9/9L
hematopoiesis**



Reduced proliferation
and survival of HSPCs

Somatic rescue events

**Monosomy 7
Deletion 7q**

Oncogenic event

UPD 7q

Gene correction

**SAMD9/9L LOF
mutations**

Partial compensation

Increased competitive
fitness of HSPCs

Supplemental data

Supplemental information 1. List of genes included in the myeloid NGS panel.

ANKRD26, ASXL1, ASXL2, BCOR, BCORL1, BRAF, CBL, CDKN2A, CEBPA, CKIT, CUX1, DDX41, DNMT3A, ETV6, EZH2, FLT3, GATA1, GATA2, GNAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KRAS, NF1, NPM1, NRAS, PHF6, PIGA, PRPF8, PTEN, PTPN11, RAD21, RUNX1, SAMD9, SAMD9L, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2.

Supplemental information 2. Clinical course of patients with SAMD9L germline disorder and monosomy 7

Patient 1 (D1297) was diagnosed with refractory cytopenia of childhood (RCC) at the age of six months. The boy was small for gestational age (SGA) at birth and presented with global developmental delay. A brain MRI showed cerebellar atrophy. Cytogenetics revealed a monosomy 7 and isochromosome 7 in 14 and 3 of 20 metaphases, respectively. A germline *SAMD9L* variant with a VAF of 30% (*SAMD9L* p.M89R) was detected, confirming the diagnosis of Ataxia-Pancytopenia (ATXPC) syndrome. Repeated complete blood counts (CBCs) showed mild leukopenia with severe to moderate neutropenia, mild macrocytic anemia, and varying thrombocytopenia (white blood cells (WBC) $3.92 - 6.40 \times 10^9/L$, absolute neutrophil count (ANC) $0.20 - 0.70 \times 10^9/L$, platelets (PLT) $90 - 139 \times 10^9/L$, hemoglobin (Hb) $9.5 - 13.0$ g/dL, mean corpuscular volume (MCV) 95 fL) without transfusion dependency. The patient also had hypogammaglobulinemia at diagnosis (IgG 54 mg/dL, IgM 17 mg/dL, IgA 12 mg/dL), but no susceptibility to infections. Seventeen months after diagnosis, *SAMD9L* p.M892R VAF in BM cells had slowly decreased to 14%, while the monosomy 7 clone increased, and the isochromosome 7 clone had disappeared. Thereafter, the *SAMD9L* p.M892R VAF stayed between 24% and 35%, while monosomy 7 was detected with a clone size ranging between 40% and 75% of interphases. At the age of 3.3 years, the patient developed severe necrotizing granulomatous lymphadenitis (ANC at the time $0.57 \times 10^9/L$) with the need for surgical intervention, prompting the indication for HSCT. Successful allogeneic HSCT from an HLA-identified matched unrelated donor (MUD) after conditioning with treosulfan, thiotepa, and fludarabine and serotherapy with anti-thymocyte globulin (ATG) was performed at the age of 3.8 years. The patient remained well and alive 1.8 years after HSCT.

Patient 2 (D1300) was diagnosed with RCC at the age of five months. Monosomy 7 was noted one month after diagnosis, and a germline *SAMD9L* variant (*SAMD9L* p.P1349L) with a VAF of 38% was detected. The girl was born SGA and had a cleft lip and palate. At diagnosis of RCC, she presented with pancytopenia (WBC $3.90 \times 10^9/L$, ANC $0.39 \times 10^9/L$, PLT $3 \times 10^9/L$, Hb 6.7 g/dL, MCV 75 fL (after transfusion)) and transfusion dependency for red blood cells and platelets. Immunological work-up revealed NK cell and B cell deficiency (CD19+ B-cells 5%, abs. $0.18 \times 10^9/L$; CD16+ CD56+ CD3- cells 2 %, abs. $0.07 \times 10^9/L$) and hypogammaglobulinemia (IgG 138 mg/dl, IgM 9 mg/dl, IgA 32 mg/dl, IgG <15 U/ml). At the age of 9 months, the CBC improved spontaneously (WBC $5.3 \times 10^9/L$, ANC $1.43 \times 10^9/L$, PLT $53 \times 10^9/L$, Hb 11.8 g/dL). At the age of 15 months, the ANC decreased and the patient received a 12-day treatment course with G-CSF for isolated neutropenia. Subsequent BM analyses showed an increase in cellularity, marked dysplasia, and an excess of blasts consistent with MDS-EB. The patient received an allogeneic HSCT from an HLA-identical matched unrelated donor after conditioning with fludarabine, thiotepa, melphalan, and a serotherapy with anti-thymocyte globulin and rituximab. She developed acute respiratory distress syndrome, veno-occlusive disease with liver failure and died from complications in remission of MDS-EB at the age of 21 months.

Patient 3 (GR012) presented with an infection and pancytopenia (WBC $3.14 \times 10^9/L$, ANC $0.35 \times 10^9/L$, PLT $12 \times 10^9/L$, Hb 8 g/dL, MCV 80 fL) at the age of four months. He was subsequently diagnosed with RCC. Further genetic work-up revealed monosomy 7 in 21 of 30 BM cell metaphases (FISH analysis: 37% of interphases) and a germline *SAMD9L* variant with a VAF of 34% (*SAMD9L* p.T1474P). He had normal immunoglobulin levels (IgG 812 mg/dL, IgM 250 mg/dL, IgA 89 mg/dL) and no prior susceptibility to infections. Nine months from diagnosis, the patient's platelet count and hemoglobin concentration had improved spontaneously (WBC $7.70 \times 10^9/L$, ANC $0.38 \times 10^9/L$, PLT $179 \times 10^9/L$, Hb 11.7 g/dL) and the germline *SAMD9L* variant had decreased to a VAF of 13%. Fourteen months from diagnosis, the patient was well without severe infections, the ANC had slightly increased to 0.50 G/L, and monosomy 7 was only present in 9 of 25 BM cell metaphases (FISH analysis: 34% of interphases). Subsequently, the patient withdrew from scheduled BM surveillance. At the age of 4.0 years (3 years and 7 months after diagnosis), the boy was alive and well with a normal CBC (WBC $6.01 \times 10^9/L$, ANC $2.15 \times 10^9/L$, PLT $207 \times 10^9/L$, Hb 12.3 g/dL, MCV 84 fL).

Patient 4 (B063) was diagnosed with RCC at the age of 7 months. His CBC at diagnosis showed moderate neutropenia, thrombocytopenia, and normocytic anemia (WBC $7.10 \times 10^9/L$, ANC $0.71 \times 10^9/L$, PLT $107 \times 10^9/L$, Hb 8.0 g/dL, MCV 78 fL). Due to a positive family history with a germline *SAMD9L* mutation in the older brother (RCC with monosomy 7) and father (healthy, normal CBC), genetic testing was performed and confirmed the familiar germline *SAMD9L* p.R986H variant with a VAF of 50%. The patient had normal immunoglobulin levels (IgG 661 mg/dL, IgM 810 mg/dL, IgA 650 mg/dL) and no susceptibility to infections. Although the first karyotype at diagnosis was normal, monosomy 7 was detected in 7% of interphases by FISH analysis three months later. Six months after diagnosis, two additional missense variants *SAMD9L* p.Y900C and *SAMD9L* p.C228Y were noted with a VAF of 4% and 27%, respectively. Cytogenetics showed a normal karyotype at this time point. Concomitantly, the hemoglobin concentration and WBC normalized, while the thrombocytopenia without transfusion dependency persisted (WBC $12.4 \times 10^9/L$, ANC $3.97 \times 10^9/L$, PLT $101 \times 10^9/L$, Hb 12.1 g/dL, MCV 85 fL). The BM morphology showed normal cellularity with low-grade dysplasia of myelopoiesis and erythropoiesis and reduced megakaryopoiesis. Platelet counts finally normalized 14 months after diagnosis. At last follow-up 4 years and 5 months after diagnosis, the patient was alive and had a normal CBC.

Patient 5 (KM) was born at 30 weeks of gestation after a triplet pregnancy. She was found to have nephrocalcinosis due to prematurity and multiple infantile hemangiomas on her back. Due to hydrocephalus, she received a ventriculoperitoneal shunt at the age of 6 months. Her CBC showed variable severe neutropenia, macrocytosis, and normal platelets (WBC $3.3 - 4.5 \times 10^9/L$, ANC $0.165 - 0.50 \times 10^9/L$, PLT $170 \times 10^9/L$, Hb 12.3 g/dL, MCV 92 fL). The immunological work-up indicated B cell and natural killer (NK) cell deficiency (CD19+ cells 0.6%, abs. $0.03 \times 10^9/L$; CD16+ CD56+ CD3- cells 0.5 %, abs. $0.02 \times 10^9/L$) and hypogammaglobulinemia (IgG 310 mg/dL, IgM 13 mg/dL, IgA 16 mg/dL). She received regular immunoglobulin substitution, which was tolerated poorly. For persistent neutropenia, she received her first BM examination at the age of 17 months. Histopathology was compatible with RCC. Cytogenetics showed monosomy 7 in all 15 metaphases (FISH analysis: 52% of interphases) and further genetic work-up revealed a germline *SAMD9L* p.A1195V variant with a VAF of 25%. Although the size of the monosomy 7 clone decreased over time and the *SAMD9L* VAF increased to 44% at the age of 4 years, the myeloid NGS panel revealed a new likely pathogenic *RUNX1* p.D198G variant (VAF 7%) and a variant of unknown significance in *EZH2* (c.1672+3_1672+4del, VAF 6%) indicating clonal evolution. HSCT from an HLA-identical matched unrelated donor was employed following a conditioning regimen with treosulfan, thiopeta, and fludarabine and a serotherapy with ATG at the age of 4.4 years and close to 3 years after diagnosis. At last follow-up 3.5 years after diagnosis and 6 months after HSCT, the patient was alive and well.

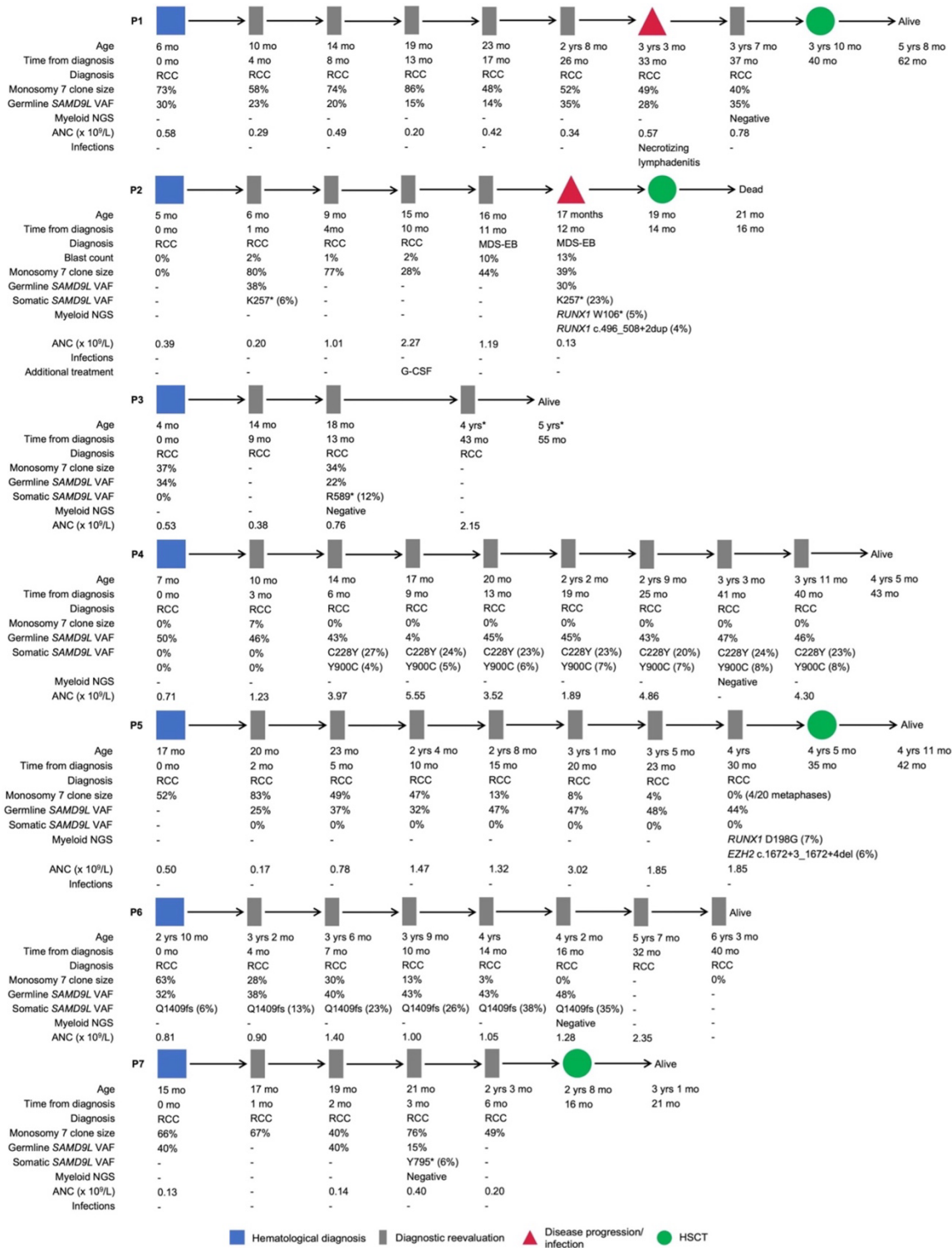
Patient 6 (CZ132) was diagnosed with RCC at the age of 2.8 years. Her CBC showed moderate neutropenia, moderate thrombocytopenia, and macrocytic anemia (WBC $4.1 \times 10^9/L$, ANC $0.81 \times 10^9/L$, PLT $46 \times 10^9/L$, Hb 11 g/dL, MCV 94 fL). There was mild B cell deficiency (CD19 12%, abs. $0.35 \times 10^9/L$; CD3 78%, abs. $2.25 \times 10^9/L$; CD3+HLADR+ 0,8%, abs. $0.02 \times 10^9/L$; CD3neg16,56+ 8,6%, abs. $0.25 \times 10^9/L$; CD4 43%, abs. $1.24 \times 10^9/L$; CD8 32%, abs. $0.92 \times 10^9/L$) and mild hypogammaglobulinemia (IgG 603 mg/dl, IgM 64 mg/l, IgA 29 g/l). Monosomy 7 was detected in 20 of 23 BM cell metaphases (FISH analysis: 63% of interphases) at diagnosis. A germline *SAMD9L* p.R1281W mutation with a VAF of 32% and a somatic *SAMD9L* p.Q1409Tfs*49 variant with a VAF of 6% were identified shortly after. BM examination 4 and 7 months after diagnosis showed persistent monosomy 7, but 10 months after diagnosis, cytogenetics revealed monosomy 7 in only 7 of 20 metaphases, while the germline and somatic *SAMD9L* VAF had increased to 43% and 26%, respectively. Subsequently, cytogenetic studies repeatedly indicated a normal karyotype, and the CBC normalized over time. At last follow-up 40 months after diagnosis, the patient was alive with a normal CBC and normal karyotype.

Patient 7 (A146) is a girl diagnosed with RCC and monosomy 7 (in 17 of 20 BM cell metaphases) at the age of 15 months. CBC at diagnosis showed leukopenia, severe neutropenia, moderate thrombocytopenia, and normocytic anemia (WBC $2.54 \times 10^9/L$, ANC $0.25 \times 10^9/L$, PLT $79 \times 10^9/L$, Hb 7.7 g/dL, MCV 89 fL). Lymphocyte phenotyping showed B cell and NK cell deficiency. The further work-up also revealed moderate hypogammaglobulinemia (IgG 273 mg/dL IgA 23 g/l, IgM 28 mg/dL). The girl displayed a failure to thrive and big eye bulbs. Genetic testing identified a germline *SAMD9L* p.D1034Y variant with a VAF of 40%. Six months after diagnosis, a somatic *SAMD9L* p.Y795* resulting in a stop codon was detected with a VAF of 7%. In the further course, the monosomy 7 clone was ranging between 40% and 76% in interphase nuclei. However, almost 12 months after diagnosis, the CBC still showed persistent leukopenia (WBC $3.75 \times 10^9/L$) and severe neutropenia (ANC $0.14 - 0.40 \times 10^9/L$). Therefore, allogeneic HSCT from an HLA-identical MUD after conditioning with treosulfan, thiotepa, and fludarabine and a serotherapy ATG was successfully performed 21 months after RCC diagnosis at the age of 2.7 years.

Patient characteristics		Genetic characteristics		
No. (UPN)	Sex	Germline genotype (VAF)	Somatic <i>SAMD9L</i> mutation (VAF)	Somatic cancer gene mutation (VAF), ACMG variant interpretation
P1 (D1297)	M	<i>SAMD9L</i> c.2675T>G, p.M892R (30%)	No	No
P2 (D1300)	F	<i>SAMD9L</i> c.4046C>T, p.P1349L (38%)	<i>SAMD9L</i> c.768dup, p.K257* (23%)	<i>RUNX1</i> c.317G>A, p.W106* (5%), P; <i>RUNX1</i> c.496_508+2dup (4%), LP
P3 (GR012)	M	<i>SAMD9L</i> c.4420A>C, p.T1474P (34%)	<i>SAMD9L</i> c.1765C>T, p.R589* (12%)	No
P4 (B063)	M	<i>SAMD9L</i> c.2957G>A, p.R986H (50%)	<i>SAMD9L</i> c.2699>G, p.Y900C (8%); c.683G>A, p.C228Y (26%); c.3562C>T, p.R1188* (4%)	No
P5 (KM)	F	<i>SAMD9L</i> c.3584C>T, p.A1195V (25%)	No	<i>RUNX1</i> c.593A>G, p.D198G (7%), LP; <i>EZH2</i> c.1672+3_1672+4del (6%), VUS
P6 (CZ132)	F	<i>SAMD9L</i> c.3841A>T, p.R1281W (32%)	<i>SAMD9L</i> c.4224dupA, p.Q1409Tfs*49 (35%)	No
P7 (A146)	F	<i>SAMD9L</i> c.3100G>T, p.D1034Y (40%)	<i>SAMD9L</i> c.2385C>A, p.Y795* (6%)	No

Supplemental Table S1. Genetic findings of all patients with *SAMD9L* germline disorder

Abbreviations: UPN: unique patient number. VAF: variant allele frequency. ACMG: American College of Medical Genetics. P: pathogenic. LP: likely pathogenic. VUS: variant of unknown significance.



Supplemental Figure S1. Clinical course of patients with germline SAMD9L disorders.

Abbreviations: VAF: Variant allele frequency. NGS: next-generation sequencing. ANC: absolute neutrophil counts. Yrs: years. Mo: months. RCC: refractory cytopenia of childhood. MDS-EB: myelodysplastic syndrome with excess of blasts. *Patient 3 was withdrawn from further BM sampling.