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ARTICLE

Disease characteristics and outcomes of acute myeloid leukemia in germline *RUNX1* deficiency (Familial Platelet Disorder with associated Myeloid Malignancy)

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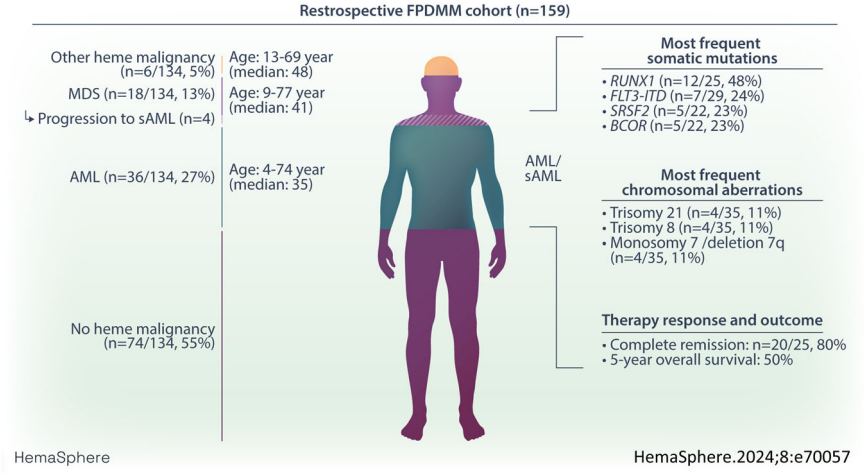
Graphical Abstract



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Disease characteristics and outcomes of acute myeloid leukemia in germline *RUNX1* deficiency (Familial Platelet Disorder with associated Myeloid Malignancy)

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Abstract

Familial Platelet Disorder with associated Myeloid Malignancy (FPDMM, FPD/AML, *RUNX1*-FPD), caused by monoallelic deleterious germline *RUNX1* variants, is characterized by bleeding diathesis and predisposition for hematologic malignancies, particularly myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Clinical data on FPDMM-associated AML (FPDMM-AML) are limited, complicating evidence-based clinical decision-making. Here, we present retrospective genetic and clinical data of the largest cohort of FPDMM patients reported to date. We describe 159 European patients (from 94 families) of whom 134 were evaluable for the development of malignant disease. Sixty developed a hematologic malignancy (44.8%), most frequently AML (36/134, 26.9%) or MDS (18/134, 13.4%). Somatic alterations of *RUNX1* by gene mutation (48%) and chromosome 21 aberrations (14.3%) were the most common somatic genetic aberrations in FPDMM-AML, followed by *FLT3-ITD* mutations (24.1%). Somatic *RUNX1* and *FLT3-ITD* mutations were not detected in FPDMM-associated MDS, suggesting important contributions to leukemic transformation. Remission-induction chemotherapy resulted in complete remission in 80% of FPDMM-AML patients with a 5-year overall survival (OS) of 50.4%. Survival outcome was non-inferior compared to a large cohort of newly diagnosed adult *RUNX1*-mutated AML (5-year OS 36.6%, $p = 0.5$), with relatively infrequent concurrent adverse risk somatic aberrations (*ASXL1* mutation, monosomal karyotype, monosomy 5/del 5q) in FPDMM-AML. Collectively, data support the notion that step-wise leukemic evolution in FPDMM is associated with distinct genetic events and indicate that a substantial subset of FPDMM-AML patients achieves prolonged survival with conventional AML treatment, including allogeneic stem cell transplant. These findings are anticipated to inform personalized clinical decision-making in this rare disorder.

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INTRODUCTION

Familial Platelet Disorder with associated Myeloid Malignancy (FPDMM, OMIM 601399, also known as FPD/AML or *RUNX1*-FPD) is a rare autosomal-dominantly inherited disease, caused by germline *RUNX1* variants. More than 20 years after its initial discovery,[1] a total of 259 FPDMM families had been reported worldwide in 2021.[2] Patients typically suffer from a bleeding diathesis, caused by (mild) quantitative and/or qualitative platelet defects.[3] Moreover, FPDMM confers a predisposition to developing hematologic malignancies, most commonly myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).[4] In addition, and possibly related to malignancy development, a higher rate of clonal hematopoiesis of undetermined potential (CHIP) is found in these patients, with a lower median age of onset compared to the general population.[5–7] The lifetime risk of developing myeloid malignancies is estimated to be around 44% based on retrospective case series of families with FPDMM,[8] but non-myeloid hematologic malignancies have also been recurrently reported.[4,9] Hematologic malignancy may develop at any life stage from childhood to (advanced) adulthood.[4,9]

Myeloid malignancy with germline predisposition was formally recognized as a separate disease entity in the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia, with germline *RUNX1* variants as one of the subcategories.[10] The presence of (likely) pathogenic germline variants in predisposition genes like *RUNX1* bears implications for the management of patients with AML or MDS, particularly in genetic counseling and clinical decision-making regarding allogeneic stem cell transplant (SCT).[11–16] As included in the ELN2022 guideline, germline predisposition should be considered in all patients with hematologic malignancies.[16] Thus, FPDMM patients should be distinguished clinically from AML or MDS patients with somatic *RUNX1* variants. In most cohorts, 8%–10% of *RUNX1*-mutated AML represents a germline *RUNX1* variant,[17–20] although this frequency was reported to range from 0% to 30% in other cohorts.[21,22] The recent discovery of somatic exonic deletions,[23] which also occur as germline variants, complicates accurate assessment of disease prevalence.

Current understanding of the clinical course of FPDMM has been largely based on case reports, case series, and literature reviews. These reports have not yet resulted in aggregated descriptions of the clinical course of hematologic malignancy in the context of FPDMM. Therefore, treatment responses and outcomes of AML in these patients remain largely elusive. In recent years, a prospective natural history study has been initiated by the National Institute of Health (NIH) and the first data were published recently.[6,9] Additionally, the *RUNX1* database (*RUNX1*db) has been established, and the first reports of the cohort within this database were published.[2,7] In the future, these studies will address outstanding questions in the field of FPDMM disease course. Here, within the network of the European Hematology Association Specialized Working Group (EHA-SWG) on Germline Predisposition for Blood Cancer, we aimed to accumulate real-world patient data from FPDMM patients to complement other initiatives. We describe data from the largest cohort of FPDMM patients published to date and begin to unveil the clinical characteristics and course of AML occurring in the context of germline *RUNX1* deficiency.

METHODS

Data collection

Investigators, (pediatric) hematologists, and clinical geneticists in the network of the European Hematology Association (EHA) specialized working group (SWG) Genetic Predisposition to Blood Cancer were approached to contribute to this study if they had previously been in contact with any of the initiators of this study on research into germline *RUNX1* deficiency or care for FPDMM patients. A structured digital request was sent for anonymized clinical data from research-consented FPDMM patients. Clinical data were retrieved from local electronic health records and/or published papers, digitally aggregated and curated. All medical histories and diagnostic test results were acquired locally. Results from locally performed Sanger sequencing or Next Generation Sequencing (NGS) on diagnostic material in a clinical or research setting were obtained, including lists of analyzed genes. For all patients whose data were shared, written informed consent was locally obtained to share and publish anonymized clinical data, or local legal regulations allowed the sharing and publishing of these data. The protocol for obtaining anonymized clinical data from different centers was approved by Hannover Medical School Committee (10408_BO_K_2022, June 2022).

RUNX1 variant classification and interpretation

Germline *RUNX1* variants were classified using updated *RUNX1*-specific curation guidelines,[24,25] by two independent reviewers. A point system in the curation guidelines allowed for the allocation of variants into one of the five categories: benign, likely benign, variant of uncertain significance, likely pathogenic, or pathogenic. Patients with a variant of uncertain significance or a (likely) pathogenic variant were included in further analyses, whereas patients with (likely) benign variants were excluded. The germline status of *RUNX1* variants was validated locally.

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On behalf of European Hematology Association Specialized Working Group Genetic Predisposition to Blood Cancer (Rare diseases)
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Data analysis, matched controls, and visualization

Statistical analyses were performed using GraphPad Prism version 9.3.1. Descriptive statistics were used to describe cohort demographics, germline variant data, and disease characteristics. Quantitative data were tested for significance using an unpaired *t*-test, while the χ^2 test was used to test the significance of qualitative data. Overall survival and relapse-free survival were calculated using Rstudio. A historical *RUNX1*-mutated AML control cohort was developed by combining available anonymized data from two recent phase-3 HOVON clinical trials.[26,27] Nearest-neighbor propensity score matching was performed to select an age-matched and sex-matched subcohort of HOVON controls for FPDMM-associated AML (selected for high-intensity induction chemotherapy) in a 5:1 ratio. Data visualization was performed using GraphPad Prism version 9.3.1, R version 4.2.1., and Adobe Illustrator version 25.1.

RESULTS

Germline variant curation and cohort selection

A request for anonymized clinical data of FPDMM patients was sent to 33 clinical departments and research groups associated with European academic institutions. Anonymized data from the electronic health records of 161 patients from 95 families with germline *RUNX1* variants were retrospectively collected via 17 contributing groups in 10 countries (the Netherlands, the United Kingdom, Germany, France, Belgium, Sweden, Norway, Spain, Italy, and Slovenia). From these, 160 patients harbored a single germline *RUNX1* variant, and one patient harbored 2 germline *RUNX1* variants, amounting to a total of 162 variants. The number of unique variants present in this cohort was 73, as some variants were identified in multiple patients (either in family members or in unrelated patients). *RUNX1* germline variant types included single nucleotide variants (SNVs) with various consequences (i.e., missense, nonsense, splice site variants), small insertions and deletions (indels) leading to frameshift, and copy number variants (large deletions and duplications) varying in size from one exon to the entire *RUNX1* gene and including one intronic deletion (Figure 1A).

All genetic variants were centrally reviewed and classified separately using the updated *RUNX1* classification guidelines established by the Clingen Myeloid Malignancy Variant Curation Expert Panel.[24,25] Detailed variant data are available in Supporting Information S1: Table 1. This analysis revealed high percentages of pathogenic (P) or likely pathogenic (LP) variants (58.6% and 30.9%, respectively), with a small proportion of variants of uncertain significance (VUS) or likely benign (LB) variants (8.6% and 1.9%, respectively) (Figure 1B). The relatively small proportion of VUS and LB variants is most likely caused by the request for clinical data of FPDMM patients, thus enriching the data for P and LP variants. Missense variants contained the highest proportion of VUS (35%), indicative of challenges in accurate classification (Figure 1C) and highlighting the necessity for functional assays.[28,29]

All 14 patients carrying a VUS displayed at least one of the FPDMM phenotypic criteria as defined in the original *RUNX1* classification guidelines (thrombocytopenia, platelet ultrastructural and/or functional defects, hematologic malignancy)[24] and were included in the analyses. Thrombocytopenia was reported in 12 patients, functional platelet defects were identified in six patients, and four patients were reported to have developed a hematologic malignancy. Two patients carrying solely an LB variant were excluded from further analyses. The patient harboring two variants (i.e., BQ-II-1) carried the LB variant p.(Gly87Cys) and P variant p.(Arg166*). It is unknown whether the variants were located *in cis* or *in trans*.

Cohort description

The exclusion of patients based on germline variant curation resulted in a cohort of 159 patients with a variant classified as P, LP, or VUS (Figure 2A). Of these patients, 35 were confirmed to have been previously published.[29–40] The cohort consisted of 84 males and 75 females from 94 families (Figure 2B). The age at which germline variants were identified was highly variable with a range from 1 month to 83 years old (median 33.5 years) (Figure 2C), indicative of a varying diagnostic delay. A similar age distribution was observed for index patients, that is, first identified patients within a family (range 1–83 years; median 34 years). The large variation in age at germline diagnosis might reflect the varying clinical penetrance and disease course of FPDMM symptoms that warrant genetic testing,[9] as well as the lack of definitive international consensus on diagnostic guidelines. Detailed patient information is included in Supporting Information S1: Table 2.

Platelet defects in FPDMM

Thrombocytopenia of varying severity was detected by local laboratories in 113 out of 127 patients (89.0%) for whom data were available. Data on functional platelet tests were reported for 36 patients, with 34 (94.4%) showing varying degrees of one or more defects in the functional testing of platelets. For 26 patients, a defect in aggregation in response to one or more agonists was reported. Decreased ATP and/or ADP release was specified for 21 patients, while defective content and/or release of alpha granules and/or dense granules was reported for three patients. Prolonged closing time in a Platelet Function Assay (PFA) was reported for five patients. In four patients, an unspecified functional defect was reported. Platelet counts at the time of functional testing were not available. Misdiagnosis of (familial) thrombocytopenia as storage pool disease or idiopathic thrombocytopenic purpura was reported for five and two patients, respectively, but available data were insufficient to determine frequencies of misdiagnosed patients or diagnostic delay this had potentially resulted in.

Hematologic malignancies in FPDMM

Data on hematologic malignancy development were available for 134 patients, of whom 60 had indeed developed hematologic malignancy (44.8%) at the time of or prior to data sharing. AML was the most common hematologic malignancy (26.9%, 36/134), followed by MDS (13.4%, 18/134) (Figure 3A). Of the 18 MDS cases, four evolved into secondary AML (sAML). Other reported malignancies included T-cell acute lymphoblastic leukemia (T-ALL) in two patients, chronic myelomonocytic leukemia (CMML) in one patient, and primary myelofibrosis (PMF) in three patients (of whom one evolved into MDS). Notably, although one case of myeloid proliferative neoplasm (MPN) without further specification has been described in FPDMM,[4] primary myelofibrosis (PMF) has not been reported before. This expands on the notion that germline *RUNX1* deficiency might confer a broader predisposition to hematologic malignancies rather than strictly to AML and MDS, albeit with substantially variable risk per malignancy type. Considering all types of hematologic malignancies in this cohort, no correlation was found between the occurrence of malignancy and the *RUNX1* variant type (Figure 3B), a topic of debate in the literature.[4,41]

Age of malignancy onset was highly variable, ranging from 4 to 77 years (median 36.5 years) (Figure 3C). The median ages at which patients presented with AML or MDS in the cohort (35 and 40.5 years, respectively) seemed to be considerably lower than the median age at which these diseases present in the general population,

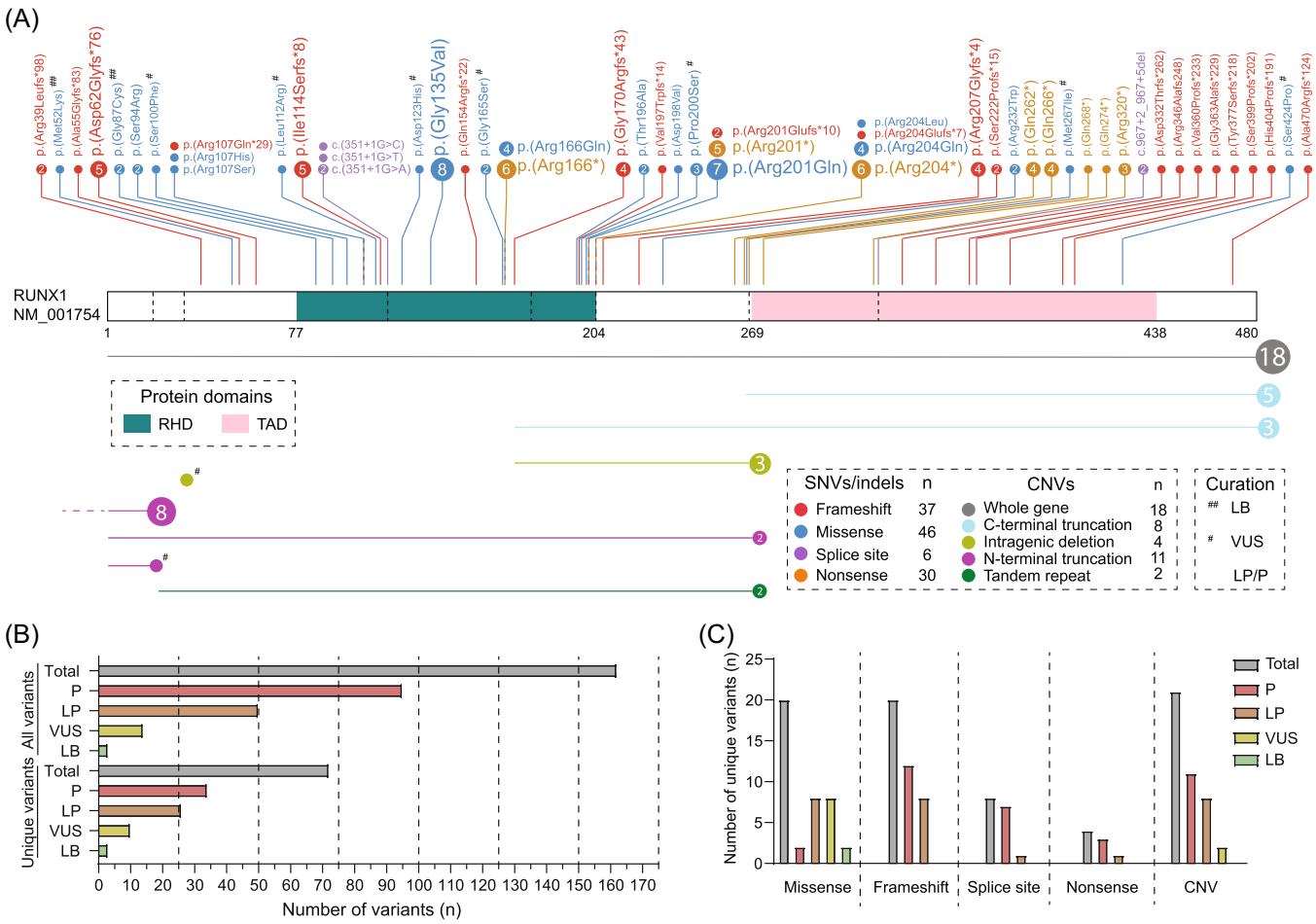

FIGURE 1 **Description and classification of germline variants. (A)** Graphical representation of germline variants identified in the cohort. Depicted is the *RUNX1C* isoform (NM_001754). SNVs and indels are indicated above the transcript; color indicates variant type, arrow indicates variant location, number indicates the number of patients in whom the variant was found. CNVs are indicated below the transcript; color indicates variant type, line indicates part of the transcript that is affected, number indicates the number of patients in whom the variant was found. A dotted line preceding exon 1 indicates (partial) deletion of the untranslated region. ## indicates likely benign variant, # indicates variants of uncertain significance, unlabeled variants were classified as pathogenic or likely pathogenic. **(B)** Classification of all variants and unique variants (i.e., without duplicates) per updated MMVCEP *RUNX1* classification guidelines.[24,25] **(C)** Distribution of pathogenicity classes in germline variant types. CNV, copy number variant; indels, insertion & deletions; LB, likely benign; LP, likely pathogenic; P, pathogenic; RHD, runt homology domain, SNV, single nucleotide variant; TAD, transactivation domain; VUS, variant of uncertain significance.

which are reportedly 69 and 77 years, respectively, in the United States.[42] No correlation was found between the age of AML onset and the type of germline *RUNX1* variant (Figure 3D). For 21 out of 30 evaluable AML patients (70%) and 11 out of 14 evaluable MDS patients (79%), the age at malignant diagnosis was identical to the age at which a germline *RUNX1* variant was identified. These numbers suggest that a considerable proportion of included patients who developed AML or MDS were diagnosed with FPDMM when malignancy developed.

Somatic mutations and chromosomal aberrations in FPDMM-associated AML and MDS

Molecular genetic data of somatic mutations were available for 30 FPDMM-associated AML (hereafter FPDMM-AML) patients and eight FPDMM-associated MDS (hereafter FPDMM-MDS) patients, describing mutations that were identified by sequencing techniques on tumor material (Figure 4A). Genes included in (panel-based) sequencing and/or Sanger sequencing varied per patient, as indicated in Figure 4A. Cytogenetic data were available for 35 FPDMM-AML patients and 10 FPDMM-MDS patients, describing chromosomal abnormalities detected by karyotyping and/or Fluorescent In Situ Hybridization techniques (Figure 4A). We compared the molecular genetic and cytogenetic data in FPDMM-AML to a large cohort of *RUNX1*-mutated AML cases ($n = 210$) included in two recent phase-3 HOVON clinical trials including newly diagnosed AML patients (age 18–65 years).[26,27] Included in these studies were patients with a diagnosis of AML, high-risk refractory anemia with excess blasts, or acute leukemia of ambiguous lineage according to WHO2008 criteria, hereafter summarized as AML. Of note, this cohort likely includes unidentified patients with *RUNX1* mutations of germline origin, typically reported to be 8%–10% in comparable cohorts.[17–20]

RUNX1 was the most frequently somatically mutated gene in FPDMM-AML (12/25, 48%), including 1 copy-neutral loss-of-heterozygosity (CN-LOH), followed by *FLT3-ITD* aberrations (7/29, 24.1%) (Figure 4A). It was unknown if somatic *RUNX1* mutations occurred *in cis* or *in trans* relative to the germline *RUNX1* variant. Somatic *RUNX1* and *FLT3-ITD* mutations were not reported in any of the 10 MDS patients. In line with this, somatic *RUNX1* mutations have

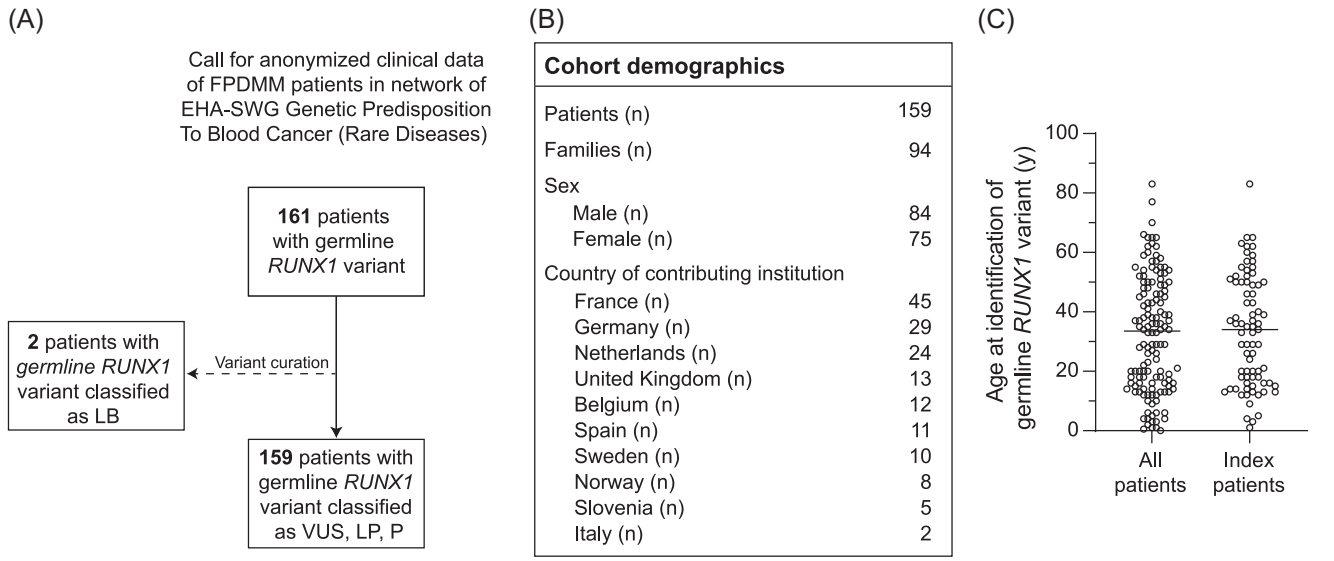

FIGURE 2 **Cohort selection and demographics**. **(A)** Consort diagram depicting cohort selection after germline *RUNX1* variant curation. **(B)** Table describing cohort demographics. **(C)** Age at identification of germline *RUNX1* variant for all patients and specifically for index patients (i.e., first identified patient within a family). Each dot represents a patient and the median age is depicted by a line. Missing data for all patients *n* = 19/159 and for index patients *n* = 14/91. B, benign, LB, likely benign; LP, likely pathogenic; P, pathogenic; VUS, variant of uncertain significance.

been identified infrequently, and *FLT3-ITD* not at all, in FPDMM-MDS in previous studies.[4,7,43] Other relatively frequently observed somatically mutated genes in this cohort of FPDMM-AML included *BCOR* (5/22, 22,7%) and *SRSF2* (5/22, 22.7%). In contrast, some other genes were notably infrequently identified to be somatically mutated, including *NPM1* (1/30, 3.3%) and *ASXL1* (1/23, 4.3%). The mutational landscape in this cohort of FPDMM-AML was generally comparable to the HOVON *RUNX1*-mutated AML cohort, with some gene-specific differences that should be cautiously interpreted due to low numbers (Figure 4B). Most strikingly, however, mutations in *ASXL1* were much less prevalent in the FPDMM-AML series compared to *RUNX1*-mutated AML patients from the HOVON cohort (57/210, 27.1%). Of note, *ASXL1* mutations are among somatic events associated with adverse risk AML in the ELN2017 and ELN2022 risk stratification.[16,44]

Chromosomal aberrations were reported in 37.1% (13/35) of AML patients and in 60% (6/10) of MDS patients (Figure 4A). Chromosome 21 (harboring the *RUNX1* gene) aberrations were among the most frequent in FPDMM-AML (5/35, 14.3%). The aberrations consisted of trisomy 21 in four cases (11.4%) and a loss of 21q in one case (2.9%). For two cases with trisomy 21, the duplicated chromosome was reported to carry the germline variant,[34] which was unknown for the remaining three cases with chromosome 21 abnormalities. In four out of five patients, chromosome 21 aberrations occurred in the absence of a somatic molecular aberration in *RUNX1*. In MDS, trisomy 21 was found in 1 out of 10 evaluable cases (10%), in which it was unknown whether the duplicated chromosome carried the germline *RUNX1* variant. Other recurrently reported chromosomal aberrations in FPDMM-AML included trisomy 8 (4/35, 11.4%) and monosomy 7 or 7q deletion (4/35, 11.4%). In the HOVON *RUNX1*-mutated AML cohort, chromosomal abnormalities were found in 105 out of 206 evaluable patients (51.0%). FPDMM-AML showed an overall trend toward lower frequencies of cytogenetic aberrations in comparison to HOVON *RUNX1*-mutated AML (Figure 4C), including monosomal karyotype (0/35, 0% vs. 12/206, 5.8%) and monosomy 5 or 5q deletions (0/35, 0% vs. 12/206, 5.8%). These aberrations represent criteria for adverse risk AML in ELN2017 and ELN2022 risk stratification.[16,44] In contrast, the frequency of trisomy 21 was lower in the HOVON *RUNX1*-mutated AML cohort (11/206, 5.3%).

Treatment response and outcome in FPDMM-associated AML

To analyze outcomes of FPDMM-AML patients with current treatment regimens, we collected therapy and treatment outcome data of FPDMM patients who had developed AML and sAML. Detailed AML data are included in Supporting Information S1: Table 3. Data on remission induction treatment were available for 31 FPDMM-AML patients. Most patients received intensive induction chemotherapy (IC, 28/31, 90.3%), whereas one patient received a hypomethylating agent (HMA), and two did not receive any induction therapy (Figure 5A). Data on the initial response to IC were available for 25 patients, of whom 20 (80%) attained complete remission (CR) or CR with incomplete hematopoietic recovery (CRi). Among these 20 patients, two were reported to have attained CRi (10%) and recovery status was not specified (CRnos) in 1 patient. Ages at diagnosis of patients who achieved CR(i) did not significantly differ from those who displayed refractory disease (RD) (Figure 5B).

Consolidation treatment data were available for 34 patients, of whom 25 received allogeneic stem cell transplant (allo-SCT, 25/34, 73.5%), three were consolidated using chemotherapy (3/34, 8.8%), and one received consolidation treatment that was not specified (1/34, 2.9%), with the remaining five patients not receiving consolidation treatment (Figure 5C). A matched unrelated donor (MUD) was the most commonly used source of stem cells for allo-SCT (15/25, 60%), whereas cord blood (CB) was used in two cases (2/25, 8%) (Figure 5C). A sibling donor was reported for six patients (24%), of which the germline *RUNX1* variant was excluded in 3/6 cases (although this was only investigated years after transplantation in one case). In two cases that received allo-SCT, donor origin could not be retrieved.

Survival data were available for 33 patients with a median age of 34 years. Treatment resulted in a 5-year overall survival (OS) of

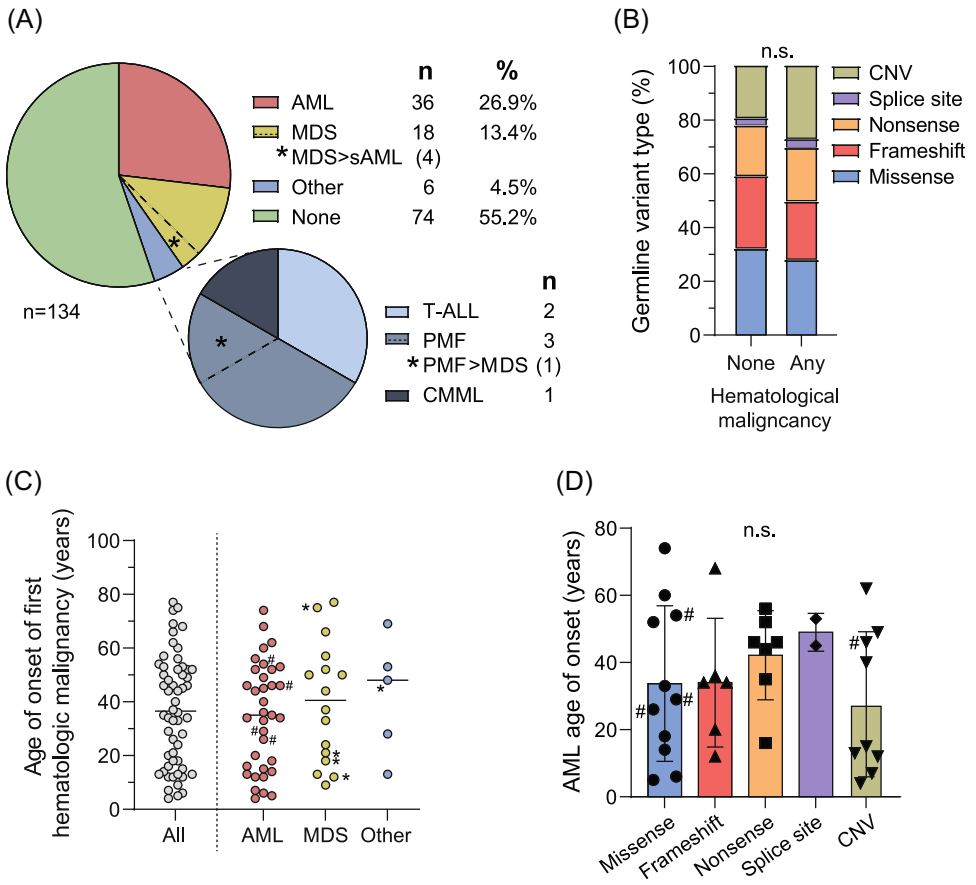

FIGURE 3 **Hematologic malignancies and correlations with germline variant types**. **(A)** Occurrence of (presenting) hematologic malignancy in 134 evaluable patients. Asterisks indicate the transformation of disease as specified in the legend. **(B)** Distribution of germline variant types in patients that developed a hematological malignancy compared to patients that did not. **(C)** Age of onset of hematologic malignancies, with dots representing individual patients and horizontal lines indicating median ages. Missing data $n = 4$ (1 AML, 2 MDS, 1 Other). **(D)** Age of primary AML onset in patients grouped per germline variant types, with dots representing individual patients and horizontal lines indicating median ages. Missing data $n = 1$ (CNV). AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; CNV, copy number variant; MDS, myelodysplastic syndrome; MDS>sAML, MDS transformed into secondary AML; n.s., not significant; PMF, primary myelofibrosis; PMF > MDS = PMF transformed into MDS; T-ALL, T-cell acute lymphoblastic leukemia; *, the transformation of malignant disease as specified in panel A; #, patients with a variant of uncertain significance.

50.4% in all evaluable patients ($n = 33$), including nine pediatric and 24 adult patients. Relapses occurred in eight out of 31 evaluable FPDMM-AML patients. Most relapses occurred within 5 years, but one patient (treated with MUD allo-SCT following IC) relapsed more than 10 years after initial diagnosis. Relapses occurred after various types of consolidation therapy; in 1/3 patients treated with chemotherapy, in 3/15 patients treated with MUD allo-SCT, in 3/6 patients treated with sibling allo-SCT (absence of germline *RUNX1* variant was established in two cases), and in 1/2 patients treated with CB allo-SCT.

We used data from the previously described HOVON studies to compare the survival data of FPDMM-AML to a historical *RUNX1*-mutated AML control cohort. The HOVON cohort included 210 *RUNX1*-mutated AML patients, with a median age of 53 years (range 18–65 years). Of these 210 patients, 160 (76%) achieved CR(i) in response to IC, comparable to response rates for FPDMM-AML (Figure 5D). Survival analysis revealed a superior survival of the FPDMM-AML cohort, with a 5-year OS of 36.6% in *RUNX1*-mutated patients in the HOVON cohort ($p = 0.05$) (Figure 5E). To correct for differences in ages between cohorts as a known prognostic factor in AML, we performed a matched analysis. In this age, sex and treatment (induction IC) matched analysis, a selection of 19 FPDMM-AML patients (median age 44 years) was compared to 95 HOVON *RUNX1*-mutated AML patients (median 48 years). In these matched cohorts, FPDMM-AML patients were more frequently treated with allo-SCT than RUNX1-mutated AML patients from HOVON, but the difference was not statistically significant ($p = 0.1$) (Table S4). After matching, FPDMM-AML patients again showed a trend toward a favorable outcome, albeit without statistical significance ($p = 0.06$) (Figure 5F).

Taken together, the data indicate that a significant subset of FPDMM-AML patients achieves prolonged survival with standard intensive AML treatment followed by consolidation strategies, including allo-SCT, and that these patients do not seem to have adverse outcomes, albeit in comparison to sporadic *RUNX1*-mutated patients.

DISCUSSION

In this work, we report on the largest cohort of FPDMM patients described to date. This concerted effort follows similar multi-center initiatives for other hematopoietic malignancy predisposition syndromes, such as germline *CEBPA* and *GATA2* deficiency,[45,46] indicating increased awareness of germline predisposition to hematologic malignancies in general.

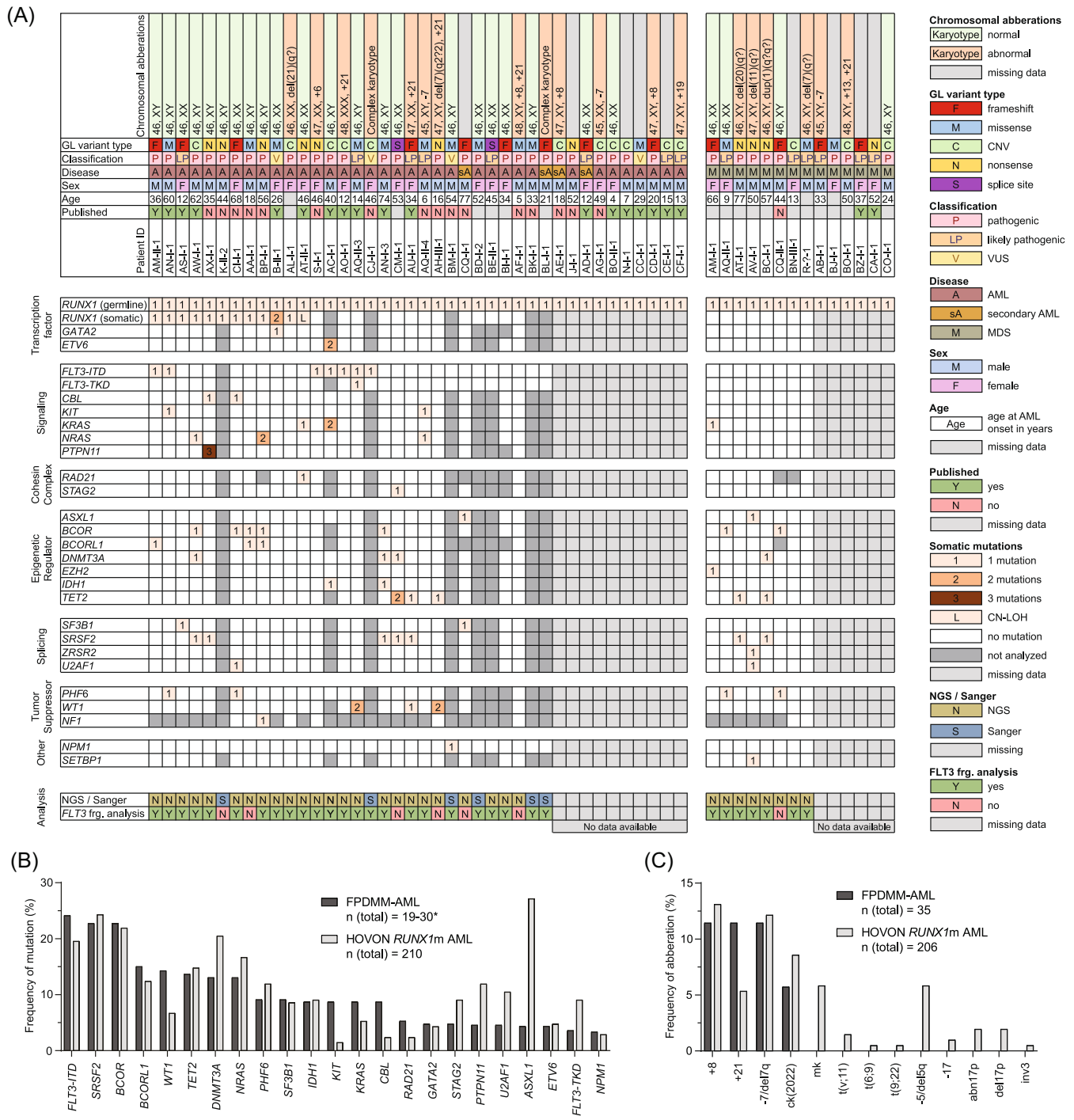

FIGURE 4 **Somatic cytogenetic and molecular aberrations in FPDMM-associated AML and MDS. (A)** Somatic cytogenetic and molecular aberrations per FPDMM patient that developed AML (FPDMM-AML) or MDS (FPDMM-MDS). Each column represents a patient. Above patient ID, rows represent patient characteristics, with cytogenetic findings in the top row (displayed as composite karyotypes). Below patient ID, each row represents a gene. Identified somatic mutations are depicted as filled cells with a number indicating the number of different mutations found in a patient in a single gene. Dark gray cells indicate a gene was not included in the analysis; light gray cells indicate missing data. Below genes, sequencing technique and additional FLT3 fragment analysis are indicated. **(B)** Comparison of somatic mutations between FPDMM-AML and *RUNX1*-mutated AML patients in HOVON data. * Number of cases in which a specific gene was included in mutational analysis, differed per gene. *NF1* was not included in this analysis due to the low number of analyses ($n = 8$). **(C)** Comparison of cytogenetic aberrations between FPDMM-AML and *RUNX1*-mutated AML patients in HOVON data. ck2022, complex karyotype according to ELN2022; CN-LOH, copy-neutral loss ofheterozygosity; mk, monosomal karyotype; *RUNX1*m, *RUNX1*-mutated.

The frequency of key FPDMM disease characteristics, such as thrombocytopenia (89.0% of patients), functional platelet defects (94.4% of patients), and development of hematologic malignancy (in 44.8% of patients) are consistent with previous reports.[4,8,9] Specifically, results on platelet defects closely resemble data presented in the prospective FPDMM natural history study,[9] in which 43/47 patients (91%) had low platelets and 18/18 patients (100%) showed one or more abnormalities in functional platelet tests. Although these data seem to confirm that

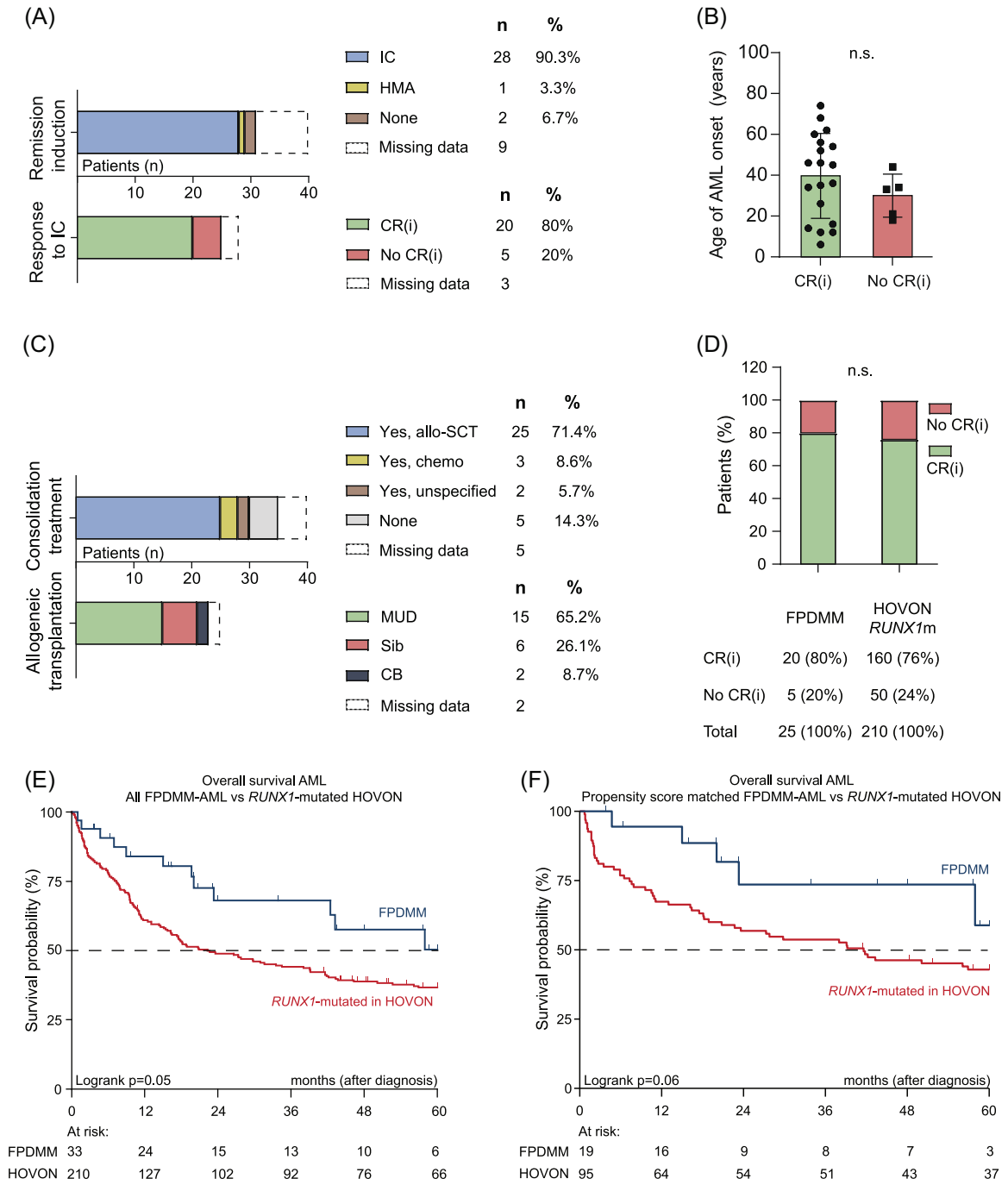

FIGURE 5 **Treatment and outcome of FPDMM-associated AML. (A)** Remission induction treatment in FPDMM-associated AML (FPDMM-AML) patients and response to intensive remission chemotherapy (IC). **(B)** Age of FPDMM-AML patients who achieved CR(i) compared to patients who did not achieve CR(i) after initial remission induction treatment. Missing data $n = 1$. **(C)** Consolidation treatment in FPDMM-AML patients. **(D)** Proportions of FPDMM-AML patients and HOVON *RUNX1*-mutated AML patients that achieve CR(i) after IC. **(E)** Overall survival of all FPDMM-AML patients compared to all HOVON patients with *RUNX1*-mutated AML. **(F)** Overall survival of a subset of FPDMM-AML patients (i.e., age at AML diagnosis between 18 and 65, treated with high-intensity chemotherapy remission induction therapy) compared to a propensity score matched cohort of HOVON patients with *RUNX1*-mutated AML. allo-SCT, allogeneic stem cell transplant; CB, cord blood; CR(i), complete remission (with incomplete recovery); HMA, hypomethylating agent; IC, intensive induction chemotherapy; MUD, matched unrelated donor; *RUNX1*m, *RUNX1*-mutated; Sib, sibling.

(mild) thrombocytopenia and thrombocytopathy are indeed hallmarks of FPDMM and present in (almost) all patients, ascertainment bias might have led to a disproportionally high number of patients with quantitative and/or qualitative platelet defects in both studies. Notably, our results confirm a heterogeneous pattern of platelet defects that precludes the use of conventional laboratory assays to establish a firm FPDMM diagnosis, stressing the requirement of molecular genetic analysis of *bona fide* germline DNA.[47,48] The clinical relevance of genetic diagnostics in a benign setting is illustrated by multiple cases of misdiagnosed platelet defects within this cohort. Besides insights into platelet defects in

FPDMM, data confirm that AML and MDS represent the most commonly occurring hematologic malignancies (initial presenting hematological malignancy in 26.9% and 13.4% of patients, respectively). Again, extrapolating these frequencies to the general FPDMM population is complicated by potential ascertainment bias and data contribution of (pediatric) hematologists who mainly treat hematological malignancies, potentially resulting in an overestimation of the incidence of MDS/AML in the general FPDMM population. Although this is highlighted by the considerable proportion of FPDMM-AML and FPDMM-MDS patients in whom a *RUNX1* variant was identified at the time of malignant diagnosis, we report a hematologic malignancy prevalence in the total cohort (44.8%) that is consistent with literature.[8] Additionally, a subset of patients in this cohort has been previously reported, potentially contributing to consistency of data, although this constituted only 22% (35/159) of patients in the current cohort.

The number of patients who developed AML or MDS in the current cohort allowed for analysis of acquired additional genetic perturbations occurring within a germline *RUNX1*-mutated background. The identification of somatic *RUNX1* mutations as the most frequent somatic genetic alteration in FPDMM-AML is consistent with existing literature,[4,7,41] further supporting the hypothesis that (further) decreasing *RUNX1* functionality is one of the mechanisms of leukemic transformation in FPDMM. It is likely that additional somatic *RUNX1* mutations result in further functional loss of RUNX1 by negatively affecting protein function, especially if both alleles would be affected (which has not formally been demonstrated in the reported FPDMM patients with somatic *RUNX1* mutations). Similarly, abnormalities of chromosome 21 potentially decrease functional *RUNX1* levels by decreasing the ratio of normal to mutated allele. This phenomenon has been previously published for 3 cases of trisomy 21 in FPDMM-AML (of which two patients were included in the current study), in which the duplicated chromosome carried the germline *RUNX1* variant.[34] Also, preclinical studies show dose-dependent effects of *RUNX1* deficiency on hematological parameters, such as reduced platelet levels and myeloid skewing.[49,50] The intricate relationship between RUNX1 function and malignant transformation is further demonstrated by recent reports of RUNX1 isoform disequilibrium involved in trisomy 21-related myeloid leukemia and FPDMM-MDS.[51,52] However, the exact mechanism by which further functional RUNX1 loss in FPDMM, either through somatic aberrations or isoform equilibrium disturbances, might drive transformation to AML, remains unclear.

Recurrent somatic mutations in genes like *FLT3*, *BCOR*, and *BCORL1* have been reported before in FPDMM-AML, as well as the relative lack of *NPM1* and *ASXL1* mutations. The much more frequent co-occurrence of *ASXL1* mutations in somatically *RUNX1*-mutated AML may point to a specific contribution of the gene in that setting. Additionally, we report a lack of *RUNX1* and *FLT3-ITD* mutations in FPDMM-MDS, suggesting that the occurrence of these mutations may play critical roles in leukemic transformation. Perturbations in these genes may thus represent biomarkers for progression, although it is unclear how imminent the risk of transformation is when these mutations are identified (in an FPDMM-MDS patient). Increased follow-up time in this cohort as well as other cohorts, preferably with sequential sequencing to determine (changes in) clone size, could offer insights into the value of such aberrations as biomarkers.

The current cohort allowed for the first analysis of aggregated FPDMM-AML treatment and outcome data. These analyses reveal response to induction treatment (CR(i)) in a substantial subset (80%) of patients, resulting in a relatively favorable 5-year OS (50.4%) in comparison to a large cohort of adult, mostly somatically, *RUNX1*-mutated AML patients treated with intensive remission induction chemotherapy. The reported FPDMM-AML patients have significantly better survival in unmatched analysis, but these data need to be interpreted with great caution given the intrinsic differences in patient and treatment characteristics between the cohorts, including younger median age and more heterogenous induction regimens in FPDMM-AML. After correcting for these differences by propensity score matching, a trend toward a favorable outcomes for FPDMM-AML remained present (albeit non-significant).

Findings may be consistent with anecdotal data on outcomes in a small set of 4 FPDMM-AML patients (100% CR, median OS 4.5 years).[53] Although caution has to be taken in the interpretation of data from small numbers of patients (inherent to the study of rare disorders), the data seem to indicate that outcomes in adult-onset FPDMM-AML are not inferior to outcomes in adult patients with *RUNX1* somatically mutated AML. While data await confirmation in future prospective data sets (for example, those currently being collected in the context of the NIH FPDMM natural history study[9]), the finding of relatively favorable outcomes in this cohort of FPDMM-AML may be related to a relative lack of concomitant somatic aberrations associated with adverse risk AML according to ELN2017 and ELN2022 genetic scores,[16,44] such as *ASXL1* mutations, monosomal karyotype, and monosomy 5 or 5q deletions. Whether the low frequency of these co-occurring aberrations is a general feature of FPDMM-AML is to be determined.

The relatively favorable outcomes in this cohort of FPDMM-AML contrast those from AML in the context of *GATA2* germline deficiency, where the majority of cases are refractory to induction,[46] and Shwachman-Diamond Syndrome (SDS), in which progression toward AML confers a dismal prognosis.[54] The nature of leukemogenic events (e.g. predominantly *TP53* mutations in SDS) likely drives the differences in outcome between these disorders. Additionally, this study suggests that FPDMM patients do not have a poor tolerance to chemotherapy, which may help explain differences in outcomes.

These novel insights into the clinical outcome of FPDMM-AML may help instruct clinical decision-making in FPDMM patients, in particular regarding the role of pre-emptive SCT in these patients. In FPDMM, questions about the indication and timing of pre-emptive SCT are complicated by incomplete penetrance of malignant transformation and the large spread of ages at which AML develops. The relatively favorable response to induction treatment and overall survival of FPDMM-AML as described herein could be used as a factor in justifying restraint regarding preemptive SCT (in comparison to predisposition disorders like *GATA2* germline deficiency and SDS). However, decisions on pre-emptive transplantation should be made on a case-by-case basis, weighing patient-specific factors against the herein-described findings, and include well-informed shared decision-making.

This collaborative multi-institutional research is an ongoing initiative by the EHA-SWG on genetic predisposition to blood cancers. Moving forward, we intend to increase the cohort size and enhance the granularity and comprehensiveness of the data to enable an even more refined description of the clinical course of FPDMM. A larger data set and increased follow-up time will further define the true incidence rates of hematologic malignancies in these patients. Additionally, larger numbers of patients and follow-up data would allow analysis of the molecular and clinical heterogeneity of FPDMM-AML patients, subgroup analysis such as pediatric versus adult patients, and identification of risk stratification that could guide therapy choices. Finally, the goal is to identify patients at high risk for leukemic transformation before hematological malignancy is diagnosed to enable the development of preventive measures rather than curative therapies.

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AUTHOR CONTRIBUTIONS

Tim Ripperger, Jörg Cammenga, Katrin Ericson, and Marc H. G. P. Raaijmakers conceptualized and designed the study and supervised Martijn P. T. Ernst. Martijn P. T. Ernst, Tim Ripperger, Jörg Cammenga, and Marc H. G. P. Raaijmakers contacted contributors. Martijn P. T. Ernst, Roger E. G. Schutgens, Bert A. Van der Reijden, Saskia M. C. Langemeijer, Rienk Y. J. Tamminga, Louise H. Hooimeijer, Marc Bierings, Kathleen Freson, Konstanze Döhner, Christian Pohlkamp, Torsten Haferlach, Panagiotis Baliakas, Jude Fitzgibbon, Inderjeet Dokal, Tor H. A. Tvedt, Kiran Tawana, Nicolas Duployez, Lise Larcher, Jean Soulier, Paolo Gresele, José M. Bastida, Tor H. A. Tvedt, Helena Podgornik, Matjaz Sever, Tim Ripperger, and Marc H. G. P. Raaijmakers extracted and contributed anonymized clinical patient data. Martijn P. T. Ernst and Tim Ripperger curated and analyzed clinical and genetic data, and curated germline *RUNX1* variants. Jurjen Versluis, Peter J. M. Valk, and Bob Löwenberg collected, curated, and managed HOVON data. Martijn P. T. Ernst, Jurjen Versluis, Peter J. M. Valk, and Marc H. G. P. Raaijmakers included and analyzed genetic and therapeutic *RUNX1*-mutated HOVON AML data, Jurjen Versluis performed a statistical comparison of FPDMM-associated AML and *RUNX1*-mutated HOVON AML data. MPTE and JV provided graphical data visualization. MPTE and Marc H. G. P. Raaijmakers wrote the manuscript, and all other authors reviewed and consented to the curated data and manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Song WJ, Sullivan MG, Legare RD, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. *Nat Genet*. 1999;23(2):166-175. doi:10.1038/13793
2. Homan CC, King-Smith SL, Lawrence DM, et al. The RUNX1 database (RUNX1db): establishment of an expert curated RUNX1 registry and genomics database as a public resource for familial platelet disorder with myeloid malignancy. *Haematologica*. 2021;106(11):3004-3007. doi:10.3324/haematol.2021.278762
3. Deuitch N, Broadbridge E, Cunningham L, Liu P. RUNX1 familial platelet disorder with associated myeloid malignancies. In: Adam MP, Feldman J, Mirzaa GM, et al., eds. *GeneReviews® [Internet]*. University of Washington, Seattle; 2024.
4. Brown AL, Arts P, Carmichael CL, et al. RUNX1-mutated families show phenotype heterogeneity and a somatic mutation profile unique to germline predisposed AML. *Blood Adv*. 2020;4(6):1131-1144. doi:10.1182/bloodadvances.2019000901
5. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood*. 2015;126(22):2484-2490. doi:10.1182/blood-2015-04-641100
6. Yu K, Deuitch N, Merguerian M, et al. Genomic landscape of patients with germline RUNX1 variants and familial platelet disorder with myeloid malignancy. *Blood Adv*. 2024;8:497-511. doi:10.1182/bloodadvances.2023011165
7. Homan CC, Drazer MW, Yu K, et al. Somatic mutational landscape of hereditary hematopoietic malignancies caused by germline variants in RUNX1, GATA2, and DDX41. *Blood Adv*. 2023;7(20):6092-6107. doi:10.1182/bloodadvances.2023010045
8. Godley LA. Inherited predisposition to acute myeloid leukemia. *Sem Hematol*. 2014;51(4):306-321. doi:10.1053/j.seminhematol.2014.08.001
9. Cunningham L, Merguerian M, Calvo KR, et al. Natural history study of patients with familial platelet disorder with associated myeloid malignancy. *Blood*. 2023;142(25):2146-2158. doi:10.1182/blood.2023019746
10. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405. doi:10.1182/blood-2016-03-643544
11. Churpek JE. Familial myelodysplastic syndrome/acute myeloid leukemia. *Best Pract Res Clin Haematol*. 2017;30(4):287-289. doi:10.1016/j.beha.2017.10.002
12. Desai AV, Perpich M, Godley LA. Clinical assessment and diagnosis of germline predisposition to hematopoietic malignancies: The University of Chicago Experience. *Front Pediatr*. 2017;5:252. doi:10.3389/fped.2017.00252
13. Godley LA, Shimamura A. Genetic predisposition to hematologic malignancies: management and surveillance. *Blood*. 2017;130(4):424-432. doi:10.1182/blood-2017-02-735290
14. Baliakas P, Tesi B, Wartiovaara-Kautto U, et al. Nordic guidelines for germline predisposition to myeloid neoplasms in adults: recommendations for genetic diagnosis, clinical management and follow-up. *Hemasphere*. 2019;3(6):e321. doi:10.1097/HS9.0000000000000321
15. Trottier AM, Godley LA. Inherited predisposition to haematopoietic malignancies: overcoming barriers and exploring opportunities. *Br J Haematol*. 2021;194(4):663-676. doi:10.1111/bjh.17247
16. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140(12):1345-1377. doi:10.1182/blood.2022016867
17. Ernst MPT, Kavelaars FG, Löwenberg B, Valk PJM, Raaijmakers MHGP. RUNX1 germline variants in RUNX1-mutant AML: how frequent? *Blood*. 2021;137(10):1428-1431. doi:10.1182/blood.2020008478
18. Mendler JH, Maharry K, Radmacher MD, et al. RUNX1 mutations are associated with poor outcome in younger and older patients with

cytogenetically normal acute myeloid leukemia and with distinct gene and microRNA expression signatures. *J Clin Oncol*. 2012;30(25):3109-3118. doi:10.1200/JCO.2011.40.6652
19. Gaidzik VI, Teleanu V, Papaemmanuil E, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. *Leukemia*. 2016;30(11):2160-2168. doi:10.1038/leu.2016.126
20. Drazer MW, Kadri S, Sukhanova M, et al. Prognostic tumor sequencing panels frequently identify germ line variants associated with hereditary hematopoietic malignancies. *Blood Adv*. 2018;2(2):146-150. doi:10.1182/bloodadvances.2017013037
21. Schnittger S, Dicker F, Kern W, et al. RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. *Blood*. 2011;117(8):2348-2357. doi:10.1182/blood-2009-11-255976
22. Simon L, Spinella JF, Yao CY, et al. High frequency of germline RUNX1 mutations in patients with RUNX1-mutated AML. *Blood*. 2020;135(21):1882-1886. doi:10.1182/blood.2019003357
23. Eriksson A, Engvall M, Mathot L, et al. Somatic exonic deletions in RUNX1 constitutes a novel recurrent genomic abnormality in acute myeloid leukemia. *Clin Cancer Res*. 2023;29(15):2826-2834. doi:10.1158/1078-0432.CCR-23-0122
24. Luo X, Feurstein S, Mohan S, et al. ClinGen myeloid malignancy variant curation expert panel recommendations for germline RUNX1 variants. *Blood Adv*. 2019;3(20):2962-2979. doi:10.1182/bloodadvances.2019000644
25. Feurstein S, Luo X, Shah M, et al. Revision of RUNX1 variant curation rules. *Blood Adv*. 2022;6(16):4726-4730. doi:10.1182/bloodadvances.2022008017
26. Löwenberg B, Pabst T, Maertens J, et al. Therapeutic value of clofarabine in younger and middle-aged (18-65 years) adults with newly diagnosed AML. *Blood*. 2017;129(12):1636-1645. doi:10.1182/blood-2016-10-740613
27. Löwenberg B, Pabst T, Maertens J, et al. Addition of lenalidomide to intensive treatment in younger and middle-aged adults with newly diagnosed AML: the HOVON-SAKK-132 trial. *Blood Adv*. 2021;5(4):1110-1121. doi:10.1182/bloodadvances.2020003855
28. Decker M, Lammens T, Ferster A, et al. Functional classification of RUNX1 variants in familial platelet disorder with associated myeloid malignancies. *Leukemia*. 2021;35(11):3304-3308. doi:10.1038/s41375-021-01200-w
29. Decker M, Agarwal A, Benneche A, et al. Validation and clinical application of transactivation assays for RUNX1 variant classification. *Blood Adv*. 2022;6(11):3195-3200. doi:10.1182/bloodadvances.2021006161
30. Marneth AE, van Heerde WL, Hebeda KM, et al. Platelet CD34 expression and α/δ-granule abnormalities in GFI1B- and RUNX1-related familial bleeding disorders. *Blood*. 2017;129(12):1733-1736. doi:10.1182/blood-2016-11-749366
31. Jongmans MCJ, Kuiper RP, Carmichael CL, et al. Novel RUNX1 mutations in familial platelet disorder with enhanced risk for acute myeloid leukemia: clues for improved identification of the FPD/AML syndrome. *Leukemia*. 2010;24(1):242-246. doi:10.1038/leu.2009.210
32. van der Crabben S, van Binsbergen E, Ausems M, Poot M, Bierings M, Buijs A. Constitutional RUNX1 deletion presenting as non-syndromic thrombocytopenia with myelodysplasia: 21q22 ITSN1 as a candidate gene in mental retardation. *Leuk Res*. 2010;34(1):e8-e12. doi:10.1016/j.leukres.2009.06.030
33. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood*. 2018;131(7):717-732. doi:10.1182/blood-2017-09-806489
34. Antony-Debré I, Duployez N, Bucci M, et al. Somatic mutations associated with leukemic progression of familial platelet disorder with predisposition to acute myeloid leukemia. *Leukemia*. 2016;30(4):999-1002. doi:10.1038/leu.2015.236
35. Fournier E, Debord C, Soenen V, et al. Baseline dysmegakaryopoiesis in inherited thrombocytopenia/platelet disorder with predisposition to haematological malignancies. *Br J Haematol*. 2020;189(4):e119-e122. doi:10.1111/bjh.16543
36. Fenwarth L, Caulier A, Lachaier E, et al. Hereditary predisposition to acute myeloid leukemia in older adults. *HemaSphere*. 2021;5(4):e552. doi:10.1097/HS9.0000000000000552
37. Engvall M, Karlsson Y, Kuchinskaya E, et al. Familial platelet disorder due to germline exonic deletions in RUNX1: a diagnostic challenge with distinct alterations of the transcript isoform equilibrium. *Leuk Lymphoma*. 2022;63(10):2311-2320. doi:10.1080/10428194.2022.2067997
38. Owen CJ, Toze CL, Koochin A, et al. Five new pedigrees with inherited RUNX1 mutations causing familial platelet disorder with propensity to myeloid malignancy. *Blood*. 2008;112(12):4639-4645. doi:10.1182/blood-2008-05-156745
39. Langabeer SE, Owen CJ, McCarron SL, et al. A novel RUNX1 mutation in a kindred with familial platelet disorder with propensity to acute myeloid leukaemia: male predominance of affected individuals. *Eur J Haematol*. 2010;85(6):552-553. doi:10.1111/j.1600-0609.2010.01513.x
40. Rio-Machin A, Vulliamy T, Hug N, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. *Nat Commun*. 2020;11(1):1044. doi:10.1038/s41467-020-14829-5
41. Förster A, Decker M, Schlegelberger B, Ripperger T. Beyond pathogenic RUNX1 germline variants: the spectrum of somatic alterations in RUNX1-familial platelet disorder with predisposition to hematologic malignancies. *Cancers*. 2022;14(14):3431. doi:10.3390/cancers14143431
42. SEER*Explorer: an interactive website for SEER cancer statistics. Surveillance Research Program, National Cancer Institute. Accessed January 4, 2024. https://seer.cancer.gov/statistics-network/explorer/
43. Sakurai M, Kasahara H, Yoshida K, et al. Genetic basis of myeloid transformation in familial platelet disorder/acute myeloid leukemia patients with haploinsufficient RUNX1 allele. *Blood Cancer J*. 2016;6(2):e392. doi:10.1038/bcj.2015.81
44. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447. doi:10.1182/blood-2016-08-733196
45. Tawana K, Wang J, Renneville A, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. *Blood*. 2015;126(10):1214-1223. doi:10.1182/blood-2015-05-647172
46. Donadieu J, Lamant M, Fieschi C, et al. Natural history of GATA2 deficiency in a survey of 79 French and Belgian patients. *Haematologica*. 2018;103(8):1278-1287. doi:10.3324/haematol.2017.181909
47. Gresele P, Harrison P, Gachet C, et al. Diagnosis of inherited platelet function disorders: guidance from the SSC of the ISTH. *J Thromb Haemostasis*. 2015;13(2):314-322. doi:10.1111/jth.12792
48. Gresele P, Bury L, Falcinelli E. Inherited platelet function disorders: algorithms for phenotypic and genetic investigation. *Semin Thromb Hemost*. 2016;42(3):292-305. doi:10.1055/s-0035-1570078
49. Soung DY, Talebian L, Matheny CJ, et al. Runx1 dose-dependently regulates endochondral ossification during skeletal development and fracture healing. *J Bone Miner Res*. 2012;27(7):1585-1597. doi:10.1002/jbmr.1601
50. Ernst MPT, Pronk E, van Dijk C, et al. Hematopoietic cell autonomous disruption of hematopoiesis in a germline loss-of-function mouse model of RUNX1-FPD. *HemaSphere*. 2023;7(2):e824. doi:10.1097/HS9.0000000000000824

51. Gialesaki S, Bräuer-Hartmann D, Issa H, et al. RUNX1 isoform disequilibrium promotes the development of trisomy 21-associated myeloid leukemia. *Blood*. 2023;141(10):1105-1118. doi:10.1182/blood.2022017619
52. Cabrerizo Granados D, Barbosa I, Baliakas P, Hellström-Lindberg E, Lundin V. The clinical phenotype of germline RUNX1 mutations in relation to the accompanying somatic variants and RUNX1 isoform expression. *Genes Chromosom Cancer*. 2023;62(11):672-677. doi:10.1002/gcc.23184
53. Lachowiez C, Bannon S, Loghavi S, et al. Clonal evolution and treatment outcomes in hematopoietic neoplasms arising in patients with germline RUNX1 mutations. *Am J Hematol*. 2020;95(11):E313-E315. doi:10.1002/ajh.25965
54. Myers KC, Furutani E, Weller E, et al. Clinical features and outcomes of patients with Shwachman-Diamond syndrome and myelodysplastic syndrome or acute myeloid leukaemia: a multicentre, retrospective, cohort study. *Lancet Haematol*. 2020;7(3):e238-e246. doi:10.1016/S2352-3026(19)30206-6