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Incidence, molecular and clinical characteristics of donor-derived hematologic malignancies after allogeneic stem cell transplantation for myeloid malignancies

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Authors contributions:

NK, FA and BF developed the research concept and provided resources, FA and BF supervised the project. TP, AB and EK performed investigations, TP, BF and FA wrote the manuscript. TP, AB, EK, CL, IR, SH, JR, RM, DJ, CW collected and assembled data, and were involved in patient care. MM and TH performed research. All authors reviewed the manuscript and approved the final version

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Abstract

Allogeneic stem cell transplantation (allo-SCT) is the only potentially curative approach for several high-risk hematologic malignancies, but high relapse rates remain a challenge. Most relapses derive from the original malignant clone or its descendants. Occasionally, secondary blood cancers arise from transplanted donor cells and are referred to as donor-derived malignancies (DDMs). To assess the incidence of DDM at our center we performed a retrospective observational study on all evaluable patients allo-transplanted for myeloid malignancies in the Dept. of SCT of UMC Hamburg-Eppendorf between 1990 and 2024. Until the end of the observation (12/2025), we observed 791 relapses after 2827 allo-SCTs (28%). For 751 (94.9%) of the relapses, material was available for detailed molecular analysis. Three myeloid malignancies (CMML, MDS, AML) were unambiguously identified as DDMs (two from matched related, one from an unrelated donor) corresponding to low frequencies of 0.1% of all transplants and 0.4% of analyzed relapses. Notably, in no case we found mutations in typical germline predisposition genes (*DDX41, RUNX1, GATA2, CEBPA*), whereas mutations associated with CHIP (*ASXL1, TET2, DNMT3A, CBL, U2AF1*) were detected in all DDMs. In conclusion, comprehensive data from this – to our knowledge largest – single-center study covering almost 3,000 consecutive transplants over a period of 35 years supports a low overall incidence of DDMs and a potential role of donor-derived clonal hematopoiesis in their development.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-SCT) remains a key therapeutic strategy and the only potentially curative approach for several hematologic malignancies. However, even after successful engraftment, relapses occur in up to 40% of cases.[1,2] Most relapses involve reemergence of the original malignant clones or more resistant or aggressive descendants, which could not be eradicated by pre-transplant treatment, conditioning and post-transplant graft-versus-leukemia (GVL) effects. Due to the prior treatment, but also to genetic predetermination, biology of relapses may also differ from that of initial disease. In rare cases, secondary hematologic malignancies arise from donor-derived hematopoietic cells after transplantation, a phenomenon referred to as donor-derived malignancy (DDM).

Though historically considered infrequent, recent data suggested that the true incidence of DDMs might have been underestimated.[2-4] In fact, published incidences of DDMs varied substantially across studies and depended on study design and diagnostic approaches. Earlier work based on large EBMT registry surveys and a survey of the Japanese national registry reported very low frequencies in the range of 0.1% of all allo-SCTs.[5-7] In contrast, two single-center cohort studies found cumulative incidences of approximately 0.5%.[8,9] Similarly, literature-based reviews and case collections implied quite diverse contributions of DDMs to overall post-transplant relapse rates between 2% and 5%.[1,10,11] Altogether, the actual incidence and relevance of DDMs after allo-SCT remains a matter of debate. At the same time, DDMs represent unique clinical and biological challenges.[2] Identifying a donor-derived leukemic clone is critical, as it influences treatment decisions, particularly because strategies aiming to boost the GVL effect may not be effective in these scenarios.

DDM typically presents with cytogenetic and phenotypic features distinct from the recipient's underlying disease and can develop months or even years after transplantation, with a median latency of 26 months.[10,12] Its pathogenesis is thought to be mediated by the presence of preexisting preleukemic clones in the donor graft and transplant-related stress factors, such as immunosuppression, bone marrow (BM) microenvironment in the recipient and pronounced replicative stress.[6,12,12] However, not all patients who were found to have received preleukemic clones develop DDM, and others showed delayed onset of disease. Together, these observations indicate that the donor-derived preleukemic or premalignant clones need to acquire additional mutations – so-called secondary hits – to transform to leukemia or other malignant disease.[14]

The prognosis remains poor, highlighting the need for timely diagnosis and individualized treatment.[11] Since germline mutations in several candidate genes have been identified as risk factors, targeted genetic testing of family donors has been proposed as a potential strategy to mitigate the risk of DDM after allo-SCT from related donors.[2]

In this retrospective observational study, we analyzed the incidence of DDMs as well as their clinical and biological data in all evaluable patients allo-transplanted for myeloid malignancies between 1990 and 2024 at our center.

Materials and Methods

Study design and patient cohort

This retrospective single-center observational study included all patients who underwent allo-SCT for myeloid malignancies at our center between 1990 and 2024. All diagnostic procedures were performed as part of routine clinical care without study-specific interventions, except for germline testing in confirmed cases of DDM. Clinical, cytogenetic, and molecular data were retrieved from patient records. Based on integrated molecular and chimerism analyses, three cases of DDM were identified and characterized (Figure 1).

Sample collection and diagnostic procedures

Bone marrow aspirates, trephine biopsies, and peripheral blood samples were analyzed using standard clinical procedures in accredited laboratories. Analyses were performed in the laboratories of the Dept. of SCT and internal (UKE) or external accredited laboratories.

Immunophenotyping and cytogenetics

Multiparameter flow cytometry (MFC) was performed on bone marrow and peripheral blood samples using myeloid panels and interpreted in conjunction with molecular and chimerism data. Cytogenetic analyses were performed in accredited laboratories, and interphase FISH was applied in selected cases, including for chimerism assessment after sex-mismatched SCT (see Supplementary Methods).

Chimerism and donor-recipient origin assays

Donor chimerism was assessed using established clinical methods. In the 1990s and early 2000s, PCR-based assays detecting short tandem repeat (STR) polymorphisms were applied. After sex-mismatched SCT, XY-FISH was performed as part of routine analysis. Subsequently, quantitative PCR[15,16] and later digital PCR techniques enumerating insertion-deletion (Indel) and sex polymorphisms were implemented for precise chimerism monitoring.[17] More recently, donor- and recipient-specific SNPs identified by amplicon-based NGS panels were used as

supportive information. Definition of full donor chimerism (DC) depended on the sensitivity of the applied methods. Particularly, in the 1990ies complete DC corresponded to ≥95% donor cells. With the introduction of qPCR and dPCR, complete DC was defined as ≥99.9% in the peripheral blood as well as purified cell populations (e.g., CD34), and as ≥99% in the bone marrow. Values below these thresholds were classified as mixed chimerism. Chimerism findings were interpreted in conjunction with molecular profiling and clinical course.

Diagnosis of DDM was based on an integrated diagnostic approach including (i) persistent complete donor chimerism, (ii) absence of the original disease-defining mutation, (iii) emergence of a new molecular or cytogenetic clone, and (iv) confirmation of donor origin by donor-specific polymorphisms. In cases of isolated extramedullary relapse with persistent full blood donor chimerism (n=18), donor origin could only be determined if tumor-specific donor-origin testing was available; these cases were classified as indeterminate (Figure 1).

Somatic mutation profiling (NGS)

Targeted next-generation sequencing (NGS) was performed using disease-adapted myeloid panels.[18] Variant annotation followed standard clinical pipelines (VAF ≥2%). Longitudinal comparison of molecular profiles enabled distinction between donor- and recipient-derived clones.[19] Detailed panel composition is provided in Supplementary Methods.

Germline predisposition analysis

Germline testing was performed only in confirmed DDM cases using clinical NGS panels that cover established predisposition genes[20] (see Supplementary Table S1).

Ethics

While not requiring formal approval, our study has respected the applying ethical rules for retrospective analyses of patient data in Germany.

Statistical analysis

Descriptive statistics were used to summarize data. Relapse incidence was estimated using cumulative incidence methods with non-relapse mortality as a competing risk. No formal hypothesis testing was performed.

Results

Incidence and classification of relapse

Between 1990 and 2024, 2827 allo-SCT were performed to treat myeloid malignancies (including AML, CML, MDS, MDS/MPN, MF) at our center. Until 12/2025, 791 patients (28%) experienced relapse (Figure 2). For 751/791 patients (94.9%) chimerism data was available at the time of relapse, whereas 40 relapses were not evaluable due to incomplete follow-up and/or missing key diagnostic data, predominantly in earlier periods of the cohort (Figure 1). Disease characteristics and stem cell sources of the evaluable cases are summarized in Tables 1-3.

Among the 751 evaluable relapses, 18 presented as isolated extramedullary disease. In these cases, systemic donor chimerism remained complete; however, tumor-specific donor-recipient origin testing was not routinely performed in extramedullary relapse cases during the study period. Therefore, these cases were considered indeterminate with regard to clonal origin. For analytical purposes, evaluable relapses were classified as recipient-derived relapse in cases showing loss of complete donor chimerism and/or molecular concordance with the primary malignancy, or as indeterminate when donor versus recipient origin could not be definitely established. In contrast, relapses were considered as donor-derived malignancy (DDM) when donor origin was unequivocally confirmed by tumor-specific testing. Three relapses were confirmed as donor-derived malignancies. The incidence of proven DDM was thus 0.4% (3/751) among evaluable relapses and 0.1% (3/2827) across the entire transplant cohort (Figure 1). Given the presence of indeterminate cases, the reported incidence represents a minimal estimate.

Case 1: Donor-derived CMML

The patient (male) was initially diagnosed with myelodysplastic syndrome, refractory cytopenia with multilineage dysplasia (MDS, RCMD) at an age of 46, which progressed to MDS with excessive blasts (MDS-EB1) approx. 18 months later. The clinical presentation included persistent anemia, thrombocytopenia, and B-symptoms. Cytogenetic testing demonstrated a normal male karyotype. The patient was treated with eight cycles of 5-azacytidine and underwent splenectomy for hypersplenism. The patient was transplanted with BM from an HLA-matched sibling two years after initial diagnosis. As early as 80 days post-SCT a novel, disease-specific donor-derived mutation in *DNMT3A*L737fs* was detected by NGS at 3.6%. Immunosuppression was rapidly tapered, and the patient received a total of three donor lymphocyte infusions (DLI) due to incomplete and fluctuating chimerism (95.9%-99.4%

according to qPCR) resulting in full donor chimerism 9 months after SCT. The *DNMT3A* clone expanded to a maximum VAF of 8.5% at one year after allo-SCT, but neither clinical nor morphological signs of disease progression were found. The *DNMT3A* clone steadily disappeared thereafter (Figure 3).

After almost three years of clinical remission with full donor chimerism, the patient was diagnosed with leukemia. BM biopsy revealed hypercellularity and trilineage dysplasia resembling CMML, while PB tests revealed anemia (Hb 7.7 g/dL), leukocytosis (28.3 g/L), and 2-5% blasts. QPCR analysis confirmed persisting complete (>99.9%) donor chimerism. NGS uncovered acquisition of a novel *PHF6*R274Q mutation (VAF 78.3%), whereas the previous *DNMT3AL737fs** mutation was not detectable anymore. 5 months after diagnosis, a second BM transplantation was performed from a mismatched unrelated donor (MMUD). The patient achieved complete remission, confirmed by a negative NGS 20 months after transplantation. Unfortunately, he developed chronic pulmonary GVHD and COPD and died of pneumogenic sepsis and multiple organ failure 2 years after the second transplant.

A schematic overview on the timeline for case 1 is provided in Figure 3.

Case 2: Donor-derived MDS

The second patient (female) was diagnosed with acute myeloid leukemia (AML, CD33+, intermediate-risk) at an age of 56. Cytogenetic analysis showed a normal female karyotype (46, XX), and FISH revealed an abnormality in the MYC gene (8q24.21). Within a few months she developed leukocytosis and underwent induction chemotherapy with DA 7+3, which lowered blast counts to 8%. A second cycle of induction chemotherapy one month later resulted in complete remission. Two months thereafter she underwent her first allo-SCT from an HLA-matched related sibling with Bu4/Flu conditioning and prophylactic administration of ATG, cyclosporine A, and mycophenolate. In view of decreased donor chimerism (PB 98.8%, BM 98.6%) two DLIs were administered 11 and 13 months after SCT, which successfully restored full donor. However, 64 months post-SCT BM examination revealed myelodysplastic neoplasia with trilineage dysplasia, >15% ringed sideroblasts, and 4% blasts; by cytogenetics a new monosomy 7 was found. White blood cell counts were 3.97 per nL, platelets 125 per nL, neutrophils 1.7 per nL, and hemoglobin 11.6 g/dL; donor chimerism was complete (>99,9%). In the following 15 months, the disease progressed with persistent dysplasia, blasts up to 7% in the BM, and transfusion dependence. PB counts worsened with anemia (Hb 8.3 g/dL) and

thrombocytosis (596/nL), and the patient developed neurological pain symptoms, presumably paraneoplastic. All measurements indicated complete donor chimerism in the BM (99.1% - 99.9%). Molecular analysis revealed an increase in donor mutations between months 6 and 15 post MDS diagnosis: *CBL*Y371H (from 2.9% in at to 42.1%), *U2AF1*Q157P (from 15.3% to 43.5%). Notably, a *DNMT3A*R882H mutation detected already early after transplantation at 3.7%, peaked at 21.6% six months after the MDS diagnosis, but disappeared with the expansion of the *CBL*Y371H / *U2AF1*Q157P clone(s). Risk stratification was IPSS-R intermediate and IPSS-M high. The diagnosis at relapse was MDS/MPN with excess blasts, of donor origin.

16 months after MDS diagnosis, the patient underwent a second allo-SCT from a mismatched unrelated male donor (PBSC, conditioned with Treosulfan/Flu/ATG). One month later, the BM was normocellular/hypocellular, with 3% blasts and mild dysplasia, primarily in megakaryopoiesis and erythropoiesis. Donor chimerism was complete (PB >99.9%, BM 99.4%), and molecular testing for *DNMT3A*, *CBL*, and *U2AF1* was negative. At the time of reporting, the patient was in remission and under observation.

The timeline for case 2 is summarized in Figure 4.

Case 3: Donor-derived AML

The patient (female) was diagnosed with primary myelofibrosis (PMF, *JAK2 V617F-positive*) at an age of 64. She presented with pancytopenia and mild hepatosplenomegaly. Cytogenetic analysis was not performed, and risk stratification revealed a median IPSS score of 2 and a high MIPSS score. Five months after diagnosis, she underwent allo-SCT from a matched unrelated male donor with Bu/Flu/ATG60 conditioning. After transplantation, she developed grade-2 acute cutaneous GVHD, she achieved complete donor chimerism (>99.9%) after six months. After 32 months, she relapsed with acute myeloid leukemia (AML, M4eo). PB analysis showed: Hb 11.0 g/dL, WBC 2.4 /nL, neutrophils 0.3 /nL, platelets 23 /nL, and 12% blasts. BM cytology revealed 16% blasts and 14% promyelocytes, while flow cytometry confirmed 19.7% blasts. Already one month later, repeat immunophenotyping revealed 42% blasts with the following profile: cyMPO+, CD117+, CD33+, CD34+, CD38+, HLA-DR+, CD64+, CD65+, CD2-. At all time points, donor chimerism remained complete (>99.9% according to qPCR and confirmed by donor-specific single nucleotide polymorphisms on NGS). Cytogenetic analysis showed 46,XY with inv(16)(p13.1q22), and FISH confirmed the MYH11::CBFB rearrangement in 82% of cells without t(15;17). Molecular testing revealed

the absence of the previously detected *JAK2*V617F mutation and was negative for the other genes tested in our panel (s. above). Together this led to the diagnosis or donor-derived acute myeloid leukemia (AML) with inv(16). The patient was treated at an external center, where she received DA 7+3 induction chemotherapy the same month. According to the external physicians, the patient achieved CR, but therapy was complicated by a left lip abscess (last update one month post chemotherapy). No further follow-up data is available for this patient.

Figure 5 schematically outlines the timeline for case 3.

Genetic analysis of DDM

For all three DDMs, amplicon NGS analysis was performed on patient (pre-SCT) and malignancy samples to detect potential mutations in the classical germline predisposition genes (*DDX41, RUNX1, GATA2, CEBPA*). Notably, no such mutation was uncovered in either of the three cases. Instead, donor-derived mutations were identified in individual cases. In case 1, a *DNMT3A L737fs** mutation emerged early after first transplantation but was no longer detectable at the time of DDM diagnosis, when a novel PHF6 R274Q mutation arose and dominated the leukemic clone (VAF 78.3%), consistent with clonal replacement. In case 2, *DNMT3A R882H* appeared early post-transplant and showed transient clonal expansion before disappearing prior to the manifestation of DDM, whereas *CBL Y371H* and *U2AF1 Q157P* demonstrated marked expansion and persisted at high VAFs (>40%), indicating their involvement in the emergence of the donor-derived leukemia. In case 3, the donor-derived AML was characterized by inv(16)(p13.1q22) with *MYH11::CBFB* rearrangement. These findings suggest clonal hematopoiesis as an important predisposing factor of DDM. In two of the three cases, cytogenetic abnormalities, namely monosomy 7 (case 2) and inv(16) (case 3), were involved in the development and/or progression of leukemia.

Discussion

Incidence and detection of DDM

Donor-derived malignancies (DDMs), including donor-derived leukemia (DDL), are a rare but clinically significant complication of allo-SCT. Their reported incidence varies across studies; based on retrospective analyses of large registries, DDM frequencies of around 0.1% of all allo-SCT were found.[5-7] However, DDM incidences, reported as cumulative incidences, were more than five times higher in several single-center cohort studies.[8,9] Moreover, recent literature-based reports suggested that DDMs may account for 2-5% of post-transplant

relapses.[1,10,11] Considering relapse occurrence in approx. 30% of patients after allo-SCT, the latter incidences would add up to 1.5% and thus be more than 10 times higher than previously anticipated. This large discrepancy has been considered to reflect underdiagnosis in earlier cohorts and the increased availability of molecular diagnostic tests in recent years. It might also result from fundamental differences in study design and methodology. Registry-based analyses provide denominator-driven estimates but may underestimate the true incidence due to limited molecular characterization, insufficient diagnostic awareness, and potential misclassification of donor-derived events as conventional relapse.[10] Importantly, our cohort represents a systematically analyzed single-center population of consecutive patients with a high proportion of molecularly evaluable relapse cases, thereby reducing both underdiagnosis and selection bias. The low number of confirmed DDMs in this setting therefore supports the notion that, although biologically relevant, donor-derived malignancies remain a rare event in routine clinical practice. At the same time, the presence of indeterminate relapse cases, even though at low frequency (7.3%; 58/791 relapse cases), suggests that the reported incidence should be interpreted as a conservative estimate.

Altogether, our study confirms that DDM represents an uncommon though clinically relevant subset of post-transplant failure. Particularly, it needs to be considered if a relapse occurs on the background of complete donor chimerism and shows novel genetic markers. Our structured cohort analysis provides a denominator-based estimate of DDM incidence and allows direct comparison with registry-derived data.

The pathogenesis of DDM is increasingly recognized as a heterogeneous, evolutionarily dynamic process shaped by donor-intrinsic genetic features, post-transplant selective pressures, and the acquisition of secondary leukemogenic events. Earlier conceptual models distinguished between two mechanisms - transmission and expansion of pre-leukemic donor clones versus de-novo transformation occurring within the altered hematopoietic microenvironment of the recipient. Accumulating genomic evidence, however, indicates that these processes frequently intersect, and DDM emerges along several biologically distinct routes rather than through a single dominant pathway.[3,21]

In recent years, germline predisposition has received growing attention as a possible mechanism of donor-derived leukemogenesis. Pathogenic variants in genes such as *DDX41*, *RUNX1*, *GATA2*, and *CEBPA* have been identified in stem cell donors, and several reports describe situations in which mutations present in donor hematopoiesis were later found in

donor-derived leukemic clones, with some donors subsequently developing hematologic disease themselves.[22-24] Those observations suggested that inherited abnormalities in donor hematopoiesis may facilitate malignant transformation in a subset of cases and led to the proposal to screen donors for the named pathogenic germline mutations, if present in patient malignancy.[2,22,23] In our study. however, no pathogenic germline variants were detected in the three observed DDM cases, indicating that inherited susceptibility (as determined by the presence of germline mutations) did not contribute to leukemic evolution in the reported cases. However, germline testing was not performed systematically across the transplant cohort and was restricted to confirmed DDM cases; therefore, no formal risk analysis regarding related donors and hereditary predisposition can be derived from our data.

A second mechanistic pathway centers on donor-derived clonal hematopoiesis (CHIP) and post-transplant clonal selection. Gondek et al[25] demonstrated that CHIP-associated mutations such as *DNMT3A* can persist for years with variable competitive fitness, while Deshmukh et al[13] proposed a multistep model in which early donor-derived CHIP clones progress only upon acquisition of additional driver mutations or under specific microenvironmental stresses. Two of the three cases in our study are quite instructive in this regard, even though there is no formal proof that the CHIP-associated mutations were already present in the donor grafts. In case 1, a donor-derived clone harboring a disease-specific mutation in *DNMT3A L737fs* peaked at a maximum VAF of 8.5% about 1 year after allo-SCT to disappear later. Instead, another clone with a typical mutation in PHD finger protein 6 (*PHF6*) took over and progressed towards AML. Similarly, in case 2 a donor-derived *DNMT3A* R882H clone persisted for several years after transplantation but became undetectable at the time of leukemic progression. Its disappearance coincided with the emergence and expansion of CBL and *U2AF1* mutations and the development of monosomy 7. Both cases thus represent constellations characteristic of clonal displacement, in which a more aggressive donor-derived subclone overtakes a less competitive early CHIP clone under selective pressure. Another mechanistic pathway involves acute chromosomal events rather than stepwise molecular evolution. Case 3 typifies this route: the patient developed donor-derived AML with inv(16), a canonical rearrangement characteristic of de-novo AML. The absence of preceding somatic mutations and the disappearance of the patient's original *JAK2*V617F mutation indicate that leukemogenesis in this case stemmed from an acute chromosomal aberration arising in donor-derived hematopoiesis. Observations by von Bonin et al[26] further highlight the contribution of post-transplant microenvironmental and immunologic factors to donor-derived clonal behavior.

Collectively, our findings underscore that donor-derived leukemogenesis does not arise through a single dominant mechanism but encompasses several biologically distinct evolutionary routes. While germline predisposition as determined by the presence of germline mutations represents one such route[27], it was not relevant in our cohort. Instead, the evolutionary trajectories observed in our three cases correspond to non-germline mechanisms described in contemporary genomic studies - namely clonal evolution of donor-derived CHIP with displacement by fitter subclones, acquisition of secondary driver mutations within donor hematopoiesis, and acute chromosomal rearrangements, potentially driven by damaged bone marrow environment.[28] This mechanistic diversity highlights the importance of integrating different diagnostic techniques to accurately determine relapse origin and clonal dynamics after allogeneic transplantation. Indeed, reliable diagnosis of DDM requires coordinated application of high-sensitivity chimerism testing, cytogenetic analysis, and NGS-based molecular profiling. Such an integrated approach allows accurate attribution of disease origin and minimizes the risk of misclassifying DDMs as relapse, thereby providing a more solid foundation for therapeutic decision-making in the post-transplant setting.

Beyond incidence, our findings support a multifactorial model of donor-derived leukemogenesis involving post-transplant clonal selection and acquisition of secondary genetic events. Although germline predisposition has been implicated in donor-derived malignancies, we did not identify known pathogenic variants in established predisposition genes in any of our cases.

Finally, the biological interplay between donor hematopoiesis and the recipient microenvironment remains incompletely understood. Experimental and clinical observations, including those from our cohort, suggest that stromal disruption, inflammation, and immune dysregulation may promote donor-derived clonal evolution. Future studies integrating serial genomic tracking with immunologic and microenvironmental profiling will be needed to clarify these interactions.

In conclusion, our study confirms the low incidence but definite risk of DDM after allo-SCT. Although two of the observed three cases occurred after SCT from matched related donors, none of the donors carried known pathogenic variants, and leukemic evolution in all three cases proceeded through alternative, non-germline related pathways putting the value of broad germline testing of related donors for risk genes under question. From a clinical standpoint, establishing donor origin through integrated chimerism, cytogenetic, and sequencing data directly influenced clinical decision-making in each case, including the indication and donor

choice for second transplantation. Our findings therefore underscore the critical role of comprehensive molecular surveillance in post-transplant care and highlight the need for broader multicenter efforts to better define the clinical and biological spectrum of this rare complication.

Limitations of our study

We only investigated relapses after allo-SCT for myeloid malignancies; our findings can therefore not be extrapolated to other conditions. The numbers of allo-SCT (2827) and DDM observed thereafter (3) remain too low to draw definite statistical conclusions. Moreover, numbers of relapses as well as DDMs might have been underestimated due to limited follow-up of patients transplanted recently.

In addition, comprehensive donor-origin assessment was feasible in 733 of 791 documented relapse cases only. Single cases of DDM might thus not have been recognized, which could have contributed to some underestimation of the DDM incidence, although the likelihood can be considered very low. Given the long observation period from 1990 to 2024, diagnostic methodologies, including chimerism techniques, evolved over time, which may have influenced the sensitivity of donor-origin detection. However, lower sensitivity of chimerism analysis would be expected to a priori result in an overestimation of DDMs. Finally, germline testing was not performed systematically across the transplant cohort but was restricted to confirmed DDM cases; therefore, no formal conclusions regarding hereditary risk or familial donor-associated predisposition can be drawn from our data.

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Tables

Table 1 Distribution of underlying hematologic diseases according to stem cell source

	1 (BM)	2 (PB)	3 (CB)	Sum
AML	54	398	1	453
CML	35	39	0	74
MDS	8	105	0	113
MDS/MPN	8	36	0	44
MF	2	105	0	107
Total	107	683	1	791

Table 2 Distribution of underlying diseases according to donor type

	Haplo/MMUD	MUD	MRD	Sum
AML	20	335	98	453
CML	2	47	25	74
MDS	2	89	22	113
MDS/MPN	3	34	7	44
MF	1	84	22	107
Total	28	589	174	791

Table 3. Underlying diseases stratified by number of allogeneic transplantations

	1	2	3	Sum
AML	372	74	7	453
CML	69	5	0	74
MDS	103	10	0	113
MDS/MPN	35	9	0	44
MF	91	16	0	107
Total	670	114	7	791

Figure legends

Figure 1 Flow diagram and classification of relapse origin after allo-SCT for myeloid neoplasia

Between 1990 and 2024, 2827 patients underwent allo-SCT at our center. Until 12/31/2025, 791 patients (28%) experienced relapse. Chimerism assessment was feasible in 751 cases, whereas 40 cases were not evaluable due to incomplete follow-up and/or missing diagnostic data. Among evaluable relapses, cases were classified as recipient-derived relapse (n = 730), donor-derived malignancy (DDM; n = 3), or indeterminate (n = 18). The incidence of proven DDM was 0.4% among evaluable relapses and 0.1% across the entire transplant cohort.

Figure 2: Cumulative incidence of relapses

The data comprises observed relapses (time-to-event analysis) in the first 10 years after allo-SCTs (n=2794) performed in patients with myeloid malignancies at our center between 1990 and 2024 (data assessment until December 31, 2025).

Figure 3. Clinical course, molecular and therapeutic features of Case 1

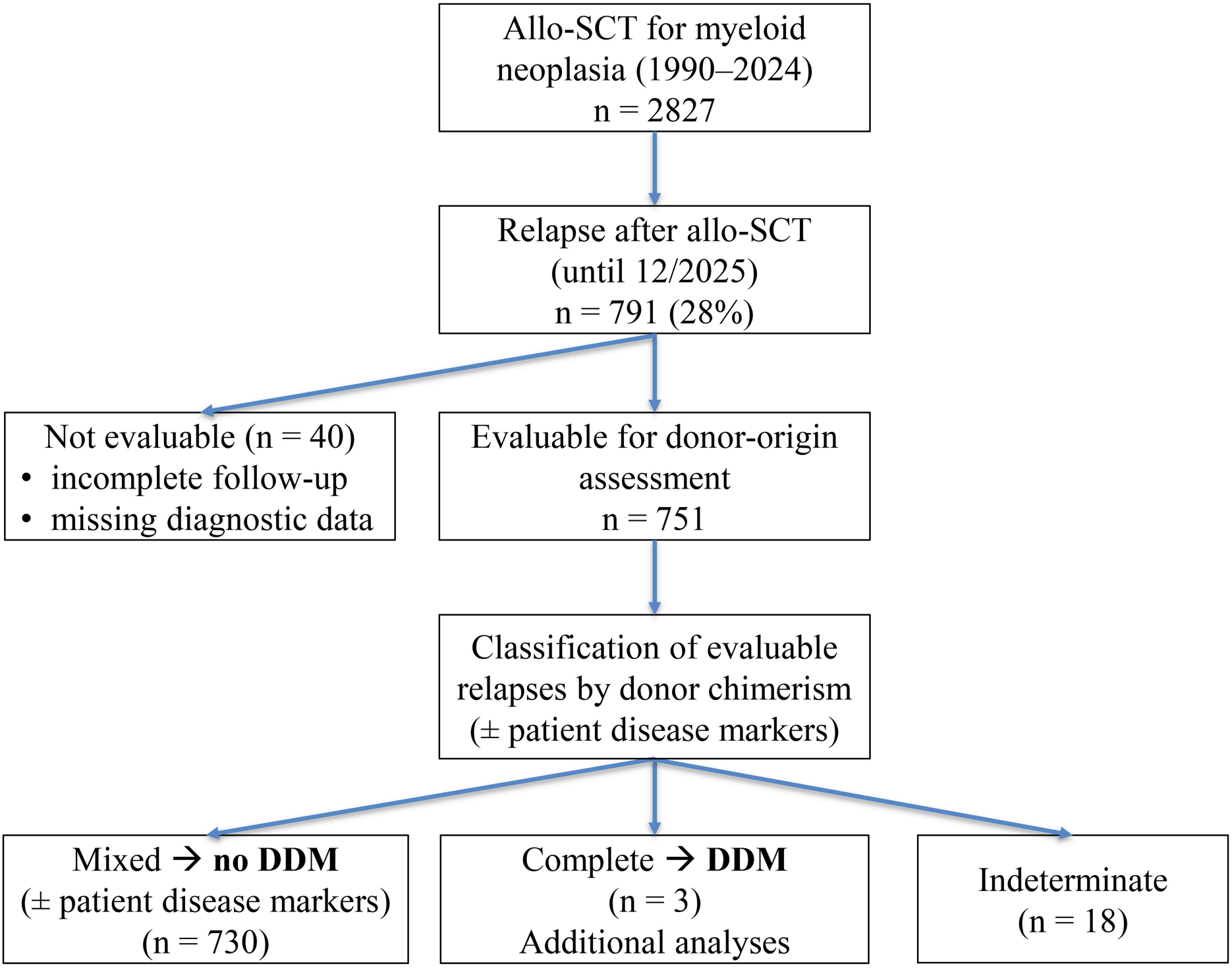
The graph summarizes the clinical history of the first DDM case. The patient was initially diagnosed with MDS, which progressed to MDS-EB1 17 months later. After allo-SCT and three rounds of DLIs the patient reached complete clinical remission with full donor chimerism. Molecular analysis revealed emergence (first detected on day 80 post-SCT) and expansion of a DNMT3AL737fs mutation (maximum VAF of 8.5%), without clinical or morphological evidence of disease progression. After three years of sustained remission the patient developed DDM-CMML. At relapse, NGS uncovered a novel PHF6 mutation (VAF 78.3%), whereas the previously detected DNMT3A mutation remained undetectable. The patient reached molecular CR after a second allo-SCT from a MMUD, but died due to transplant-related mortality (chronic pulmonary GVHD and COPD, pneumogenic sepsis).

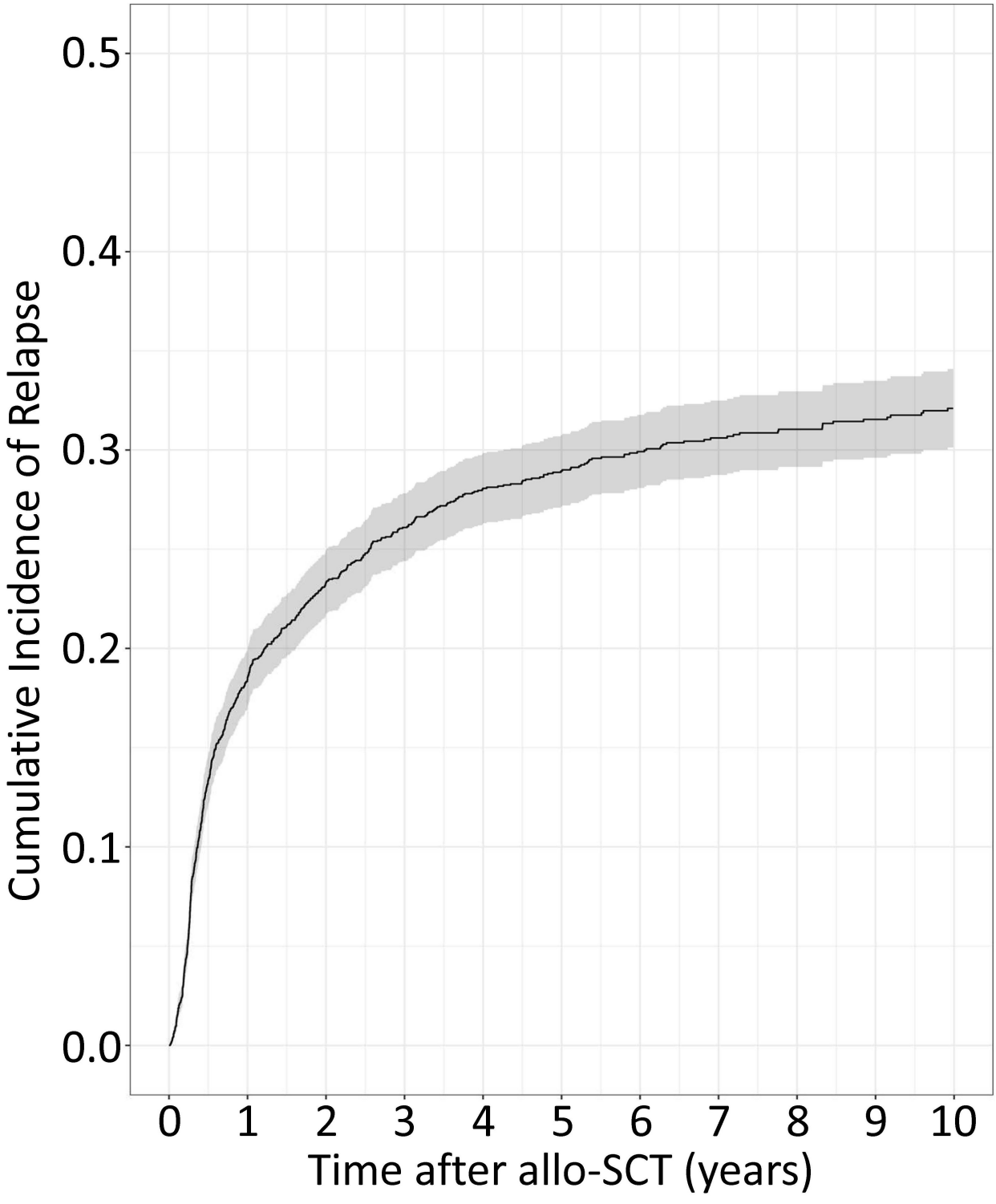
Figure 4. Clinical course, molecular and therapeutic features of Case 2

Patient 2 was first diagnosed with intermediate-risk AML and achieved complete remission after two cycles of DA 7+3, followed by allo-SCT from an MRD. Decreasing donor chimerism was corrected by two DLIs about 9-10 months post-SCT. More than 5.5 years after SCT, MDS/MPN with excess blasts and monosomy 7 was diagnosed despite complete donor chimerism and therefore defined as donor-derived. In the following year, progression (PD) with rising blasts and expansion of donor mutations CBL Y371H and U2AF1 Q157P was observed, whereas DNMT3A R882H disappeared before second SCT. A second allo-SCT from a mismatched unrelated donor was performed seven years after her first AML diagnosis. Soon thereafter, complete donor chimerism and molecular remission were confirmed; the patient remains in complete remission (CR).

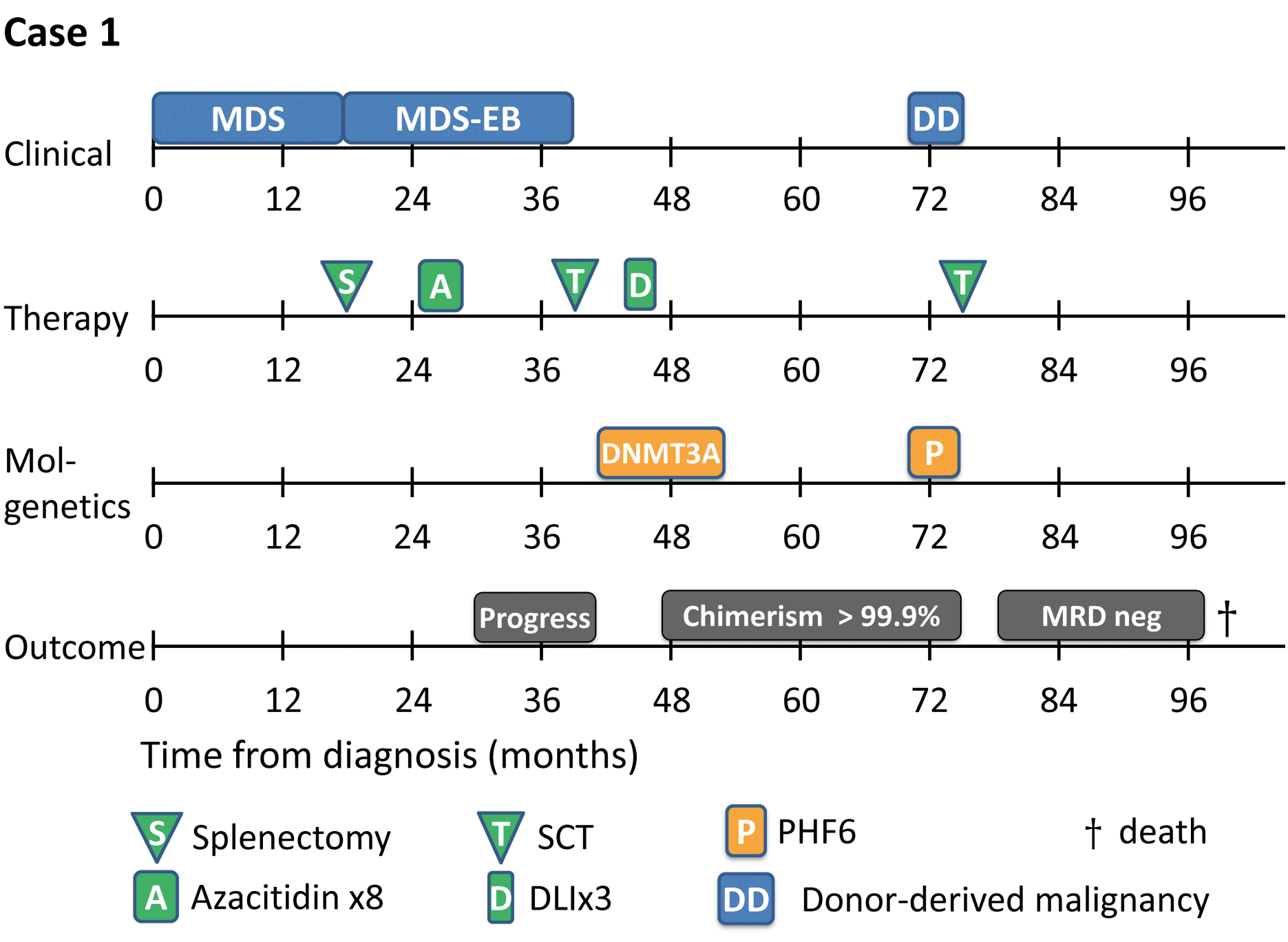
Figure 5. Clinical course, molecular and therapeutic features of Case 3

Five months after diagnosis of JAK2V617F-positive PMF, int-2 risk patient 3 underwent allo-SCT from a matched unrelated male donor. Six months post-SCT she achieved complete donor chimerism. Approx. 26 months later, she was diagnosed with AML (M4eo) presenting with 42% bone marrow blasts by immunophenotyping. Donor chimerism remained complete (>99.9%) at all time points. Cytogenetics revealed 46, XY with inv(16)(p13.1q22) and MYH11::CBFB rearrangement; JAK2 V617F was no longer detectable. Immediately, induction chemotherapy with DA 7+3 was initiated resulting in complete remission (CR), but was complicated by a lip abscess; further follow-up has not been available.

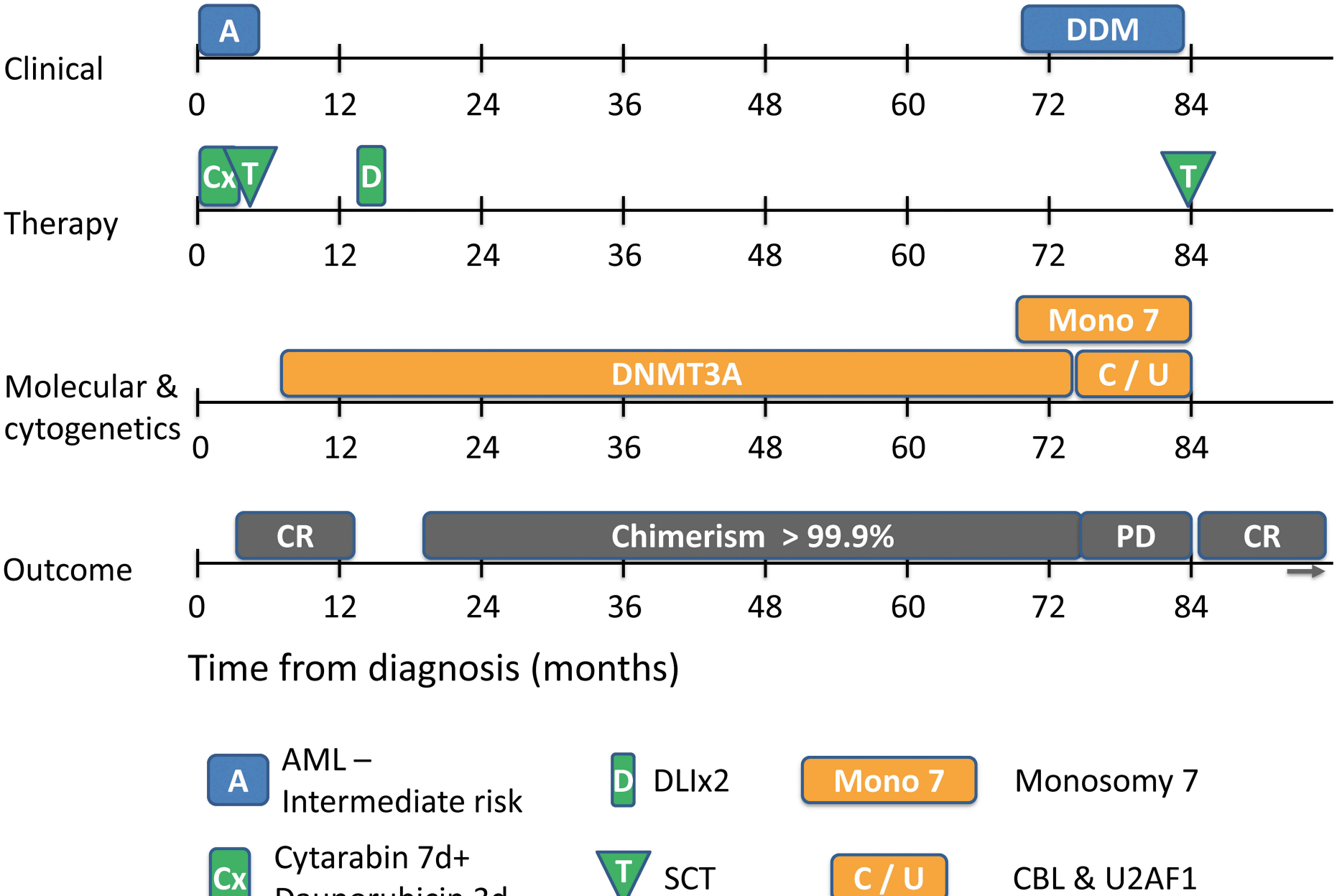

Allo-SCT for myeloid neoplasia (1990–2024) n = 2827
Relapse after allo-SCT (until 12/2025) n = 791 (28%)
Not evaluable (n = 40)
• incomplete follow-up
• missing diagnostic data
Evaluable for donor-origin assessment n = 751
Classification of evaluable relapses by donor chimerism (± patient disease markers)
Mixed → no DDM (± patient disease markers) (n = 730)
Complete → DDM (n = 3) Additional analyses
Indeterminate (n = 18)



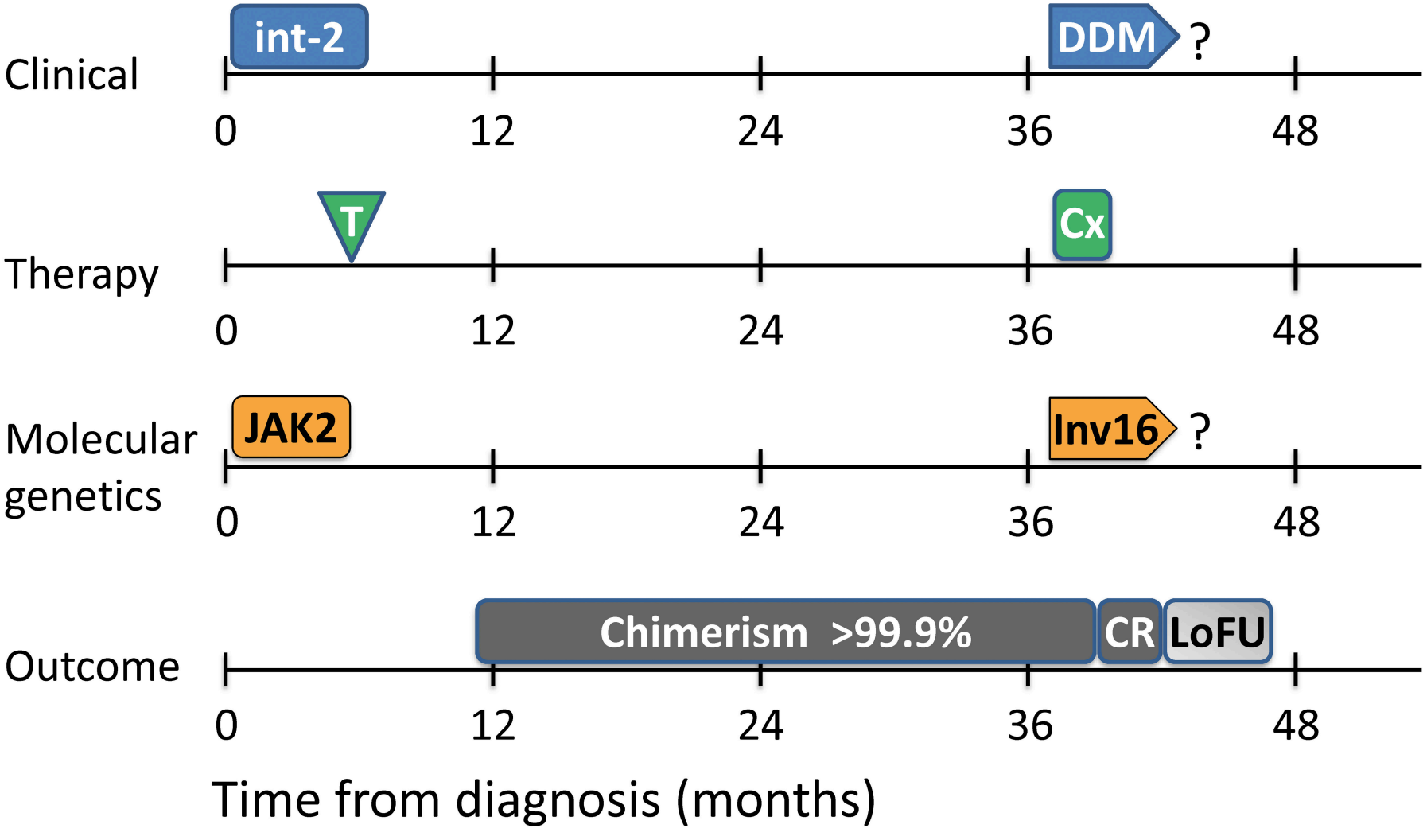
At risk	2791	1467	1187	1017	866	745	624	520	464	408	353
Events	0	497	611	672	711	726	741	750	755	760	765


Case 1
Clinical
MDS
MDS-EB
DD
0
12
24
36
48
60
72
84
96
Therapy
S
A
T
D
T
0
12
24
36
48
60
72
84
96
Mol-genetics
DNMT3A
P
0
12
24
36
48
60
72
84
96
Outcome
Progress
Chimerism > 99.9%
MRD neg
†
0
12
24
36
48
60
72
84
96
Time from diagnosis (months)
S Splenectomy
A Azacitidin x8
T SCT
D DLIx3
P PHF6
DD Donor-derived malignancy
† death

Case 2


Clinical
A
DDM
0
12
24
36
48
60
72
84
Therapy
Cx
T
D
T
0
12
24
36
48
60
72
84
Molecular &
cytogenetics
Mono 7
DNMT3A
C / U
0
12
24
36
48
60
72
84
Outcome
CR
Chimerism > 99.9%
PD
CR
0
12
24
36
48
60
72
84
Time from diagnosis (months)
A
AML –
Intermediate risk
D
DLIx2
Mono 7
Monosomy 7
Cx
Cytarabin 7d+
Daunorubicin 3d
T
SCT
C / U
CBL & U2AF1

Case 3



int-2 PMF, Intermediate risk 2

Cx Cytarabin 7d + Daunorubicin 3d

T SCT

LoFU Loss of follow-up

Supplementary Methods

Cytogenetics and fluorescence in situ hybridization (FISH)

Cytogenetic analyses to assess chromosomal aberrations were performed in accredited laboratories. In selected cases, interphase fluorescence in situ hybridization (interphase-FISH) was applied, particularly after sex-mismatched SCT. Interphase-FISH was performed on fixed cells, in which specific regions of the X and Y chromosomes were directly labeled within the nuclei. For quantification, typically 100 interphase nuclei were evaluated for female (XX) and male (XY) genotypes.

Somatic mutation profiling (NGS)

Panels contained the following genes (representing different functional groups): ASXL1, EZH2, SETBP1, EZH2 (chromatin modification), DNMT3A, IDH1, IDH2, TET2 (DNA methylation), SF3B1, SRSF2, U2AF1, ZRSR2 (RNA splicing), ETV6, TP53, RUNX1, GATA2, RAS/RAF/MEK/ERK (transcription pathways), KIT, BRAF, NRAS, KRAS (signaling pathway), and PTPN11, JAK2 exon 12, MPL, CALR exon 9, NPM1, CEBPA, CBL, IDH1, IDH2, FLT3, JAK2V617F. Variant annotation and filtering followed standard clinical pipelines, with a variant allele frequency (VAF) threshold ≥2%. Longitudinal comparison of molecular profiles at baseline and relapse enabled distinction between donor-derived and recipient-derived clones.

Germline predisposition analysis

Germline predisposition testing was not performed systematically across the transplant cohort but was conducted in the three confirmed cases of donor-derived malignancy (DDM). Testing for established predisposition genes (including DDX41, RUNX1, GATA2, CEBPA and others) was performed at the Munich Leukemia Laboratory (MLL, Munich, Germany) using validated clinical germline NGS panels (Supplementary Table S1). Analyses were conducted on DNA isolated from patients' bone marrow and/or peripheral blood samples with full donor chimerism (>99.9%) and after relapse.

Supplementary Table S1 NGS panel used to identify germline mutations

ASXL1	ASXL2	ATRX	BCOR	BRAF	CALR	CBL	CEBPA	CSF3R
CSNK1A1	CUX1	DDX41	DNMT3A	ETNK1	ETV6	EZH2	FBXW7	FLT3
GATA1	GATA2	GNB1	IDH1	IDH2	JAK2	KIT	MPL	MYD88
NF1	NOTCH1	NPM1	NRAS	PHF6	PIGA	PPM1D	PRPF8	PTPN11
RAD21	RUNX1	SETBP1	SF1	SF3A1	SF3B3	SMC1A	SMC3	SRSF2
STAG2	SUZ12	TET2	TP53	U2AF1	WT1	ZRSR2		