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Therapy-related myeloid neoplasms resembling juvenile myelomonocytic leukemia: A case series and review of the literature

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Abstract

Therapy-related myeloid neoplasms (t-MN) are a distinct subgroup of myeloid malignancies with a poor prognosis that include cases of therapy-related myelodysplastic syndrome (t-MDS), therapy-related myeloproliferative neoplasms (t-MPN) and therapy-related acute myeloid leukemia (t-AML). Here, we report a series of patients with clinical features consistent with juvenile myelomonocytic leukemia (JMML), an overlap syndrome of MDS and myeloproliferative neoplasms that developed after treatment for another malignancy.

Keywords

JMML; MDS; AML; secondary; therapy-related

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Conflict of Interest

The authors declare no conflict of interest.

Introduction

Advances in risk-stratification and treatment of pediatric malignancies have led to improved outcomes over the last several decades[1]. This increase in survivorship has been accompanied by long-term complications of therapy, with the development of a second cancer among the most feared[2]. Secondary malignant neoplasms (SMN) are defined as a histologically distinct malignant neoplasm developing at least two months after completion of therapy for the primary cancer[2]. The Childhood Cancer Survivor Study (CCSS) reported a 30-year cumulative incidence of SMN at 9.3% with a standardized incidence ratio of 6.4 compared to the general population[3]. While SMNs can occur decades after the primary cancer diagnosis, the majority of patients develop SMNs within the first 10 years[2]. SMNs account for significant morbidity in childhood cancer survivors as death rates due to SMN exceed all other causes (including disease recurrence) at 20 years of follow-up[4]. The main risk factors identified to date include chemotherapy and ionizing radiation. In particular, cytotoxic agents including alkylating agents, topoisomerase II inhibitors, antimetabolites and anti-tubulin agents, in addition to radiotherapy are associated with the occurrence of therapy-related myeloid neoplasm (t-MN)[5].

The 2016 revised World Health Organization (WHO) classification defines t-MN as a subgroup of acute myeloid leukemia (AML) that incorporates therapy-related AML (t-AML), therapy-related myeloproliferative neoplasms (t-MPN) and therapy-related myelodysplastic syndrome (t-MDS) with no major differences in outcome between the groups[6,7].

The only characterized disorder with both myelodysplastic and myeloproliferative features in children is juvenile myelomonocytic leukemia (JMML), a rare and aggressive malignancy of infants and toddlers. The median age at disease onset is approximately 2 years; however, rare late cases do occur, and patients typically present with fever, splenomegaly, thrombocytopenia, and a high circulating white blood cell (WBC) count with peripheral monocytosis[8]. Around 50% of patients also show elevated, age-corrected, hemoglobin F. The biochemical hallmark of the disease is hyperactivation of the RAS/MAPK signaling pathway[9,10]. JMML is associated with a spectrum of diverse outcomes ranging from spontaneous resolution to aggressive disease requiring allogeneic hematopoietic cell transplantation (HCT)[11]. In the following report we describe a case series of five pediatric patients with t-MN who presented with typical or atypical features of JMML.

Methods

The patients' guardians provided informed consent to this study in accordance with the Declaration of Helsinki. Clinical histories were obtained from the medical record. Genomic DNA from peripheral blood, bone marrow or buccal swabs was extracted using standard protocols. DNA samples were sequenced for clinical purposes except for one patient who received research-grade, RNASeq and whole-exome sequencing as part of their diagnostic workup (described below). Granulocyte-macrophage colony stimulating factor (GM-CSF) hypersensitivity assays were performed with peripheral blood mononuclear cells (PBMCs)

cultured in methylcellulose with increasing concentrations of human GM-CSF (R&D Systems) and colonies were enumerated after 14 days as previously described[12].

Next generation sequencing

RNA and DNA library construction for RNA and whole-exome DNA sequencing was performed according to the manufacturer's instructions using the Illumina TruSeq RNA sample preparation V2 and Nextera rapid capture exome kits, respectively. Sequencing was completed on the Illumina HiSeq 2000 according to the manufacturer's instructions. Analysis of RNA and whole-exome sequencing data, which includes mapping, coverage, and quality assessment, SNV/indel detection, tier annotation for sequence mutations, and prediction of the deleterious effects of missense mutations, has been described previously[13].

Results

Case Vignette #1

Patient was diagnosed with metastatic hepatoblastoma (HBL) with multiple pulmonary metastases at 20 months of age after presenting with tachypnea and increasing abdominal distension. She was treated per SIOPEL-4 with 12 weeks of chemotherapy followed by orthotopic liver transplant[14]. Due to the patient's high-risk disease, she was given two cycles of adjuvant chemotherapy per POG9645 which were completed 8 months after diagnosis with a total doxorubicin dose of 330 mg/m^2. Three months after finishing therapy she was found to have a hemoglobin of 7.0 g/dL with a white blood cell count of 57 × 10^9/L. The white blood cell count (WBC) increased to 160 × 10^9/L with 50% monocytes noted on the differential and hemoglobin F level of 8.5%. Bone marrow studies did not identify an increased blast population, although there was increased myelomonocytic proliferation. A complex karyotype was consistent with clonal evolution (46,XX,t(7;11)(q32;q23)[3]/45,sl, −16[5]/44,sdl1,−13[5]/42~44,sdl2,i(8)(q10),der(17)t(13;17)(q14;p13)[cp3]/44,sdl1,−10[4]). RNASeq revealed two in-frame fusions (KMT2A-AHCYL2 and PIK3CD-AKT1), as well as NRAS p.Q61K, TP53 p.E286K, MLL2 p.P511fs and MYST4 p.D1664A mutations. None of these were identified in a germline sample. After failing to respond to cytoreductive treatment with hydroxyurea and 6-mercaptopurine as well as two courses of low-dose cytarabine (40 mg/m^2 dose), she had improvement in her leukocytosis with a five-day course of high-dose cytarabine (2,000 mg/m^2 dose). While pending identification of an appropriate HCT donor, she continued to receive cytoreductive courses of high-dose cytarabine with fludarabine. Due to recurrent leukocytosis, the patient was started on decitabine. Pre-transplant evaluations revealed chronic lung and kidney changes thought to be secondary to intermittent leukostasis but showed no evidence for HBL recurrence. After two days of busulfan and rabbit anti-thymocyte globulin (ATG) as part of her umbilical cord blood transplant preparative regimen (busulfan, fludarabine, melphalan and rabbit ATG with tacrolimus starting day −1 and MMF starting day +1), she rapidly redeveloped worsening leukocytosis with a WBC peaking at 174 × 10^9/L and associated anemia, hypercalcemia, and creatinine elevation. She developed progressively worsening acute renal and hepatic failure, abdominal compartment syndrome with diffuse bowel infarction, hemorrhage, coagulopathy and severe metabolic derangements due to hyperleukocytosis. She subsequently developed

hemorrhagic and cardiogenic shock despite resuscitative efforts ultimately leading to cardiac arrest and death.

Case Vignette #2

Patient was diagnosed with pre-B-acute lymphoblastic leukemia (ALL) at 22 months of age after presenting with pallor and fatigue. Cytogenetics and fluorescent in situ hybridization (FISH) were normal and he did not have central nervous system involvement. The patient was treated per the Children's Oncology Group protocol (COG), AALL0932 and achieved minimal residual disease (MRD) negativity at the end of induction chemotherapy. His treatment course was complicated by frequent episodes of cytopenias requiring dose reductions of chemotherapy, a seizure secondary to neurotoxicity from intrathecal methotrexate, and several bacterial bloodstream infections. Three months after finishing treatment, he was noted to have a monocytosis and myeloid blasts (9%) on a routine follow-up CBC. He was found to have hepatosplenomegaly, an elevated fetal hemoglobin (3.9%) and GM-CSF hypersensitivity (see Figure 1A). Bone marrow aspirate showed less than 5% blasts and cytogenetics revealed monosomy 7. Additionally, somatic mutations in NF1 p.T676fs and NRAS p.G13R were identified. A diagnosis of therapy-related myeloid neoplasm was made, and the patient was started on azacitidine. The patient progressed after one cycle and was started on fludarabine and high-dose cytarabine. The patient's blast percentage continued to increase to 10% and acquisition of a KRAS p.G12D mutation was also identified. He then received one cycle of cytarabine, daunorubicin and etoposide and achieved a cytogenetic and molecular remission. The patient underwent allogeneic peripheral blood stem cell transplantation from a 10/12 human leukocyte antigen (HLA)-matched unrelated donor after conditioning with clofarabine, fludarabine, busulfan and rabbit ATG. His posttransplant course was complicated by acute skin and eye graft versus host disease. The patient is currently alive and well 4.5 years after HCT with no evidence of disease and full donor chimerism.

Case Vignette #3

Patient was diagnosed with B-cell ALL at 12 years of age with a chromosome 9p deletion detected on FISH. He achieved MRD negativity after induction per COG AALL1131 and completed treatment at age 15. At age 17, he developed a monocytosis with increased blasts (~11%) on bone marrow aspirate. Cytogenetics and FISH revealed monosomy 7 and molecular analyses demonstrated CBL p.R420G, RUNX1 p.L98Sfs, NRAS p.G12D, and NRAS p.G12V mutations. None of these abnormalities were present in his ALL, nor were they identified in the germline using skin fibroblasts. Hemoglobin F was not elevated. He received cytarabine, daunorubicin and etoposide without any response. He was then treated with azacitidine for two cycles but had persistence of 4–11% blasts and abnormal cytogenetics. He subsequently received decitabine and gemtuzumab. Following three cycles of this therapy, bone marrow analysis revealed MRD of 1.8% and the patient underwent evaluation for HCT. However, repeat bone marrow analysis showed progression of his disease and given the poor clinical state of the patient and the unlikely possibility of a cure, HCT was put on hold. The patient resumed palliative chemotherapy with decitabine and gemtuzumab but developed recurrent infections and frequent need for transfusions, so supportive care only measures were started. Leukocytosis and monocytosis continued to

rise slowly. He developed splenomegaly and pulmonary infiltrates that did not respond to antibiotics and as such were presumed to be malignant infiltration. He received palliative radiation therapy to the spleen for pain relieve. He died of respiratory failure at 19 months post development of treatment related malignancy.

Case Vignette #4

Patient was diagnosed with high-risk B- ALL at 3 years of age and was CNS 2a at diagnosis. He was treated on COG AALL0232 and received cranial radiation (12 Gy). Patient presented one year after completing therapy with splenomegaly, a WBC of 15.2 k/μL with a monocytosis and circulating metamyelocytes and myelocytes. Fetal hemoglobin was elevated at 6.9%. A bone marrow was performed which demonstrated 15% blasts in a background of dysgranulopoiesis. FISH studies revealed monosomy 7 and sequencing revealed an NRAS p.G12A mutation. He was treated with one cycle of AML therapy with mitoxantrone and cytarabine and then proceeded to a matched sibling donor HCT after conditioning with busulfan and cyclophosphamide. Two years post-HCT he relapsed with frank AML but achieved a morphologic remission with additional chemotherapy and then underwent a matched unrelated donor HCT after conditioning with busulfan and fludarabine. Post-transplant, he developed 2 isolated chloromas which were treated with radiation alone. He is alive and well at 17 years of age with no evidence of disease.

Case Vignette #5

Patient was diagnosed with metastatic atypical teratoid rhabdoid tumor (ATRT) characterized by biallelic inactivation of SMARCB1 at the age of 3 years. The child was treated as per the Dana-Farber Cancer Institute ATRT protocol and received chemotherapy including cisplatin (360mg/m^2), doxorubicin (480mg/m^2), cyclophosphamide (12g/m^2), etoposide (900mg/m^2), and temozolomide (2g/m^2). Following chemotherapy, the child received craniospinal radiation with boosts to the sites of intracranial disease and spinal metastatic disease. Therapy was completed at the age of 4, and the patient's ATRT was in remission, but bloodwork demonstrated persistent thrombocytopenia requiring frequent platelet transfusions. Four months after completing therapy, a peripheral blood monocytosis was noted and a bone marrow examination was performed, which showed an increase in mature monocytes without evidence of acute leukemia or a lymphoproliferative disorder. Karyotype showed 2/30 cells with t(1;10) and t(4;6) rearrangements consistent with a small clonal population. Eight months after completion of therapy, the patient underwent a repeat bone marrow aspirate and biopsy given a worsening leukocytosis with a significant peripheral blood monocytosis, mild anemia, and ongoing transfusion-dependent thrombocytopenia. The marrow showed dysplasia, 2% blasts, and 5% promonocytes. The karyotype at this time was normal, but a pathogenic KRAS p.G12D mutation was detected by next generation sequencing (NGS) The patient was found to have an elevated fetal hemoglobin (14%), but no splenomegaly was noted on exam or by abdominal ultrasound. The patient initially received a cycle of chemotherapy consisting of azacytidine, fludarabine, and high-dose cytarabine. This was followed by a cycle of venetoclax and azacytidine. The patient then received an unrelated donor HCT and post-transplant bone marrow confirmed that theirr disease was in remission with no minimal residual disease detected by flow

cytometry. The child is now approximately 2 months out from bone marrow transplant and doing well.

GM-CSF Assays

We performed a GM-CSF hypersensitivity assay with cryopreserved PBMCs from two patient samples that were available (patient #2 and patient #4). As shown in Figure 1 panel A, colony formation in the absence of GM-CSF, a typical hallmark of JMML cells, was observed.

Next generation sequencing

RNASeq and whole exome sequencing were performed on the JMML sample described in vignette 1. RNASeq revealed two novel in-frame fusions including *KMT2A-AHCYL2* and *PIK3CD-AKT1* (Figure 1B). Whole exome sequencing identified several pathogenic mutations including two in genes known to activate the RAS/MPAK pathway including *NRAS* and *MYST4*. Additional alterations included a frameshift in *MLL2* and a point mutation in *TP53* (see Table 1). There was also a heterozygous deletion across chromosome 16 involving but not limited to *CTCF* and *CREBBP*.

Discussion

Here, we describe five pediatric patients with t-MN who demonstrated features of both myelodysplasia as well as myeloproliferation (Table 1, Supplemental Table 1). None of these patients met criteria for t-AML and the presence of splenomegaly and myeloproliferation is not characteristic of t-MDS. These patients had a median latency from end of treatment to the development of a t-MN of only 8 months (range 3–36), a shorter period than reported with other t-MNs in pediatrics[15]. Similar to patients with *de novo* JMML, these five patients harbored mutations in genes encoding signaling proteins in the RAS/MAPK pathway. In-frame fusions including *KMT2A-AHCYL2* and *PIK3CD-AKT1* were detected in patient #1. *KMT2A* is frequently truncated and fused to a range of genes from other chromosomes. These fusion proteins have been observed in myeloid, lymphoid and therapy-related leukemias and it is hypothesized that the truncation and fusion of *KMT2A* independent of its fusion partner is sufficient for leukemogenesis[16,17]. Although *KMT2A* rearrangements are not classically associated with JMML, their cooperativity with RAS/MAPK pathway mutations has been well established[18–20]. RAS/MAPK pathway mutations are frequently subclonal in AML and ALL patients harboring *KMT2A* rearrangements, in contrast to patient #1 where the *NRAS* mutation was in the dominant clone with a variant allele frequency of 43%, potentially explaining the difference in presentation between acute leukemia and t-MDS/MPN[18,21]. Alternatively, the cell of origin that acquires the *KMT2A* rearrangement may contribute to the difference in clinical phenotypes. No germline mutations that predispose to the development of JMML were detected in any of the four patients who had germline material available for analysis. Patient #2, #3 and #4 received alkylating agents as part of their ALL-treatment protocols which could have predisposed to the development of monosomy 7. Patient #5 also received an alkylating agent as part of her therapy but did not have any evidence of chromosome 5 or 7 abnormalities.

We identified five previous cases of secondary chronic myelomonocytic leukemia (CMMoL), a prior name for JMML, in the literature: The earliest report dates back to 1977 by Inoue *et al.* who described a 4-year-old child who was initially treated for ALL when a gradual increase of immature monocytes and myeloblasts was noted, followed by elevation of fetal hemoglobin[22]. George *et al.* described a 10-year-old girl who developed CMMoL with t(9;11) following treatment for T-cell lymphoma[23]; additional reports described three patients who were diagnosed with CMMoL after treatment for B-ALL[24–26]. Clinical characteristics of previously reported patients are summarized in Table 2.

Different disease models of t-MNs have been proposed: (1) the induction of oncogenes in susceptible target cells during cytotoxic therapy; (2) the selection of pre-existing clones that are treatment-resistant; (3) inherited cancer predisposition; and (4) clonal hematopoiesis of indeterminate potential (CHIP) that is accelerated in the context of cytotoxic therapy[5]. Leukemias following alkylating agent therapy are often characterized by complex karyotypes with partial or complete loss of chromosome 5 and/or chromosome 7. In contrast, prior topoisomerase II inhibitor therapy is associated with *KMT2A* rearrangements in t-AML that often presents as monoblastic or myelomonocytic leukemia[27]. Previous studies found a high frequency of *TP53* mutations in t-MDS/t-AML compared to *de novo* MDS/AML, while t-AML showed a higher frequency of *FLT3*, *NRAS* and *KRAS* mutations than t-MDS. Similar to *de novo* AML, patients with t-AML are often treated based on their cytogenetic risk profile[28,29]. However, these patients are often excluded from frontline clinical trials and there are no randomized studies comparing standard AML therapy to other forms of treatment[28]. Additionally, organ dysfunction from prior therapy often complicates treatment and overall survival for patients with t-MN remains dismal.

Altogether, these ten cases of therapy-related MDS/MPN in pediatric patients with clinical and molecular features of JMML constitute a subgroup of patients with t-MN that is poorly understood. Similar to patients with t-MDS/t-AML, it is hypothesized that HCT is the only curative treatment for these patients. The ideal chemotherapy regimens indicated for these patients remains unclear but cytotoxic chemotherapy, hypomethylating agents, and MEK inhibitors are strategies that have been effective in JMML[30–32]. Similarly, busulfan, cyclophosphamide and melphalan is the standard preparative regimen in JMML and should be considered for these patients before undergoing HCT[33,34]. Further investigations are necessary to identify the optimal therapeutic regimens for this subgroup of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

List of Abbreviations:

SMN	Secondary malignant neoplasm
CCSS	Childhood Cancer Survivor Study
t-MN	Therapy-related myeloid neoplasm
WHO	World Health Organization
AML	Acute myeloid leukemia
t-AML	Therapy-related AML
MDS	Myelodysplastic syndrome
t-MDS	Therapy-related MDS
CHIP	Clonal hematopoiesis of indeterminate potential
MPN	Myeloproliferative neoplasms
ATRT	Atypical teratoid rhabdoid tumor
JMML	Juvenile myelomonocytic leukemia
WBC	White blood count
HCT	hematopoietic cell transplantation
CMMoL	Chronic myelomonocytic leukemia
GM-CSF	Granulocyte-macrophage colony stimulating factor
HBL	Hepatoblastoma
MRD	Minimal residual disease
PBMCs	Peripheral blood mononuclear cells
NGS	Next generation sequencing
FISH	Fluorescent in situ hybridization
ATG	Anti-thymocyte globulin
COG	Children’s Oncology Group
ALL	Acute lymphoblastic leukemia

HLA	Human leukocyte antigen

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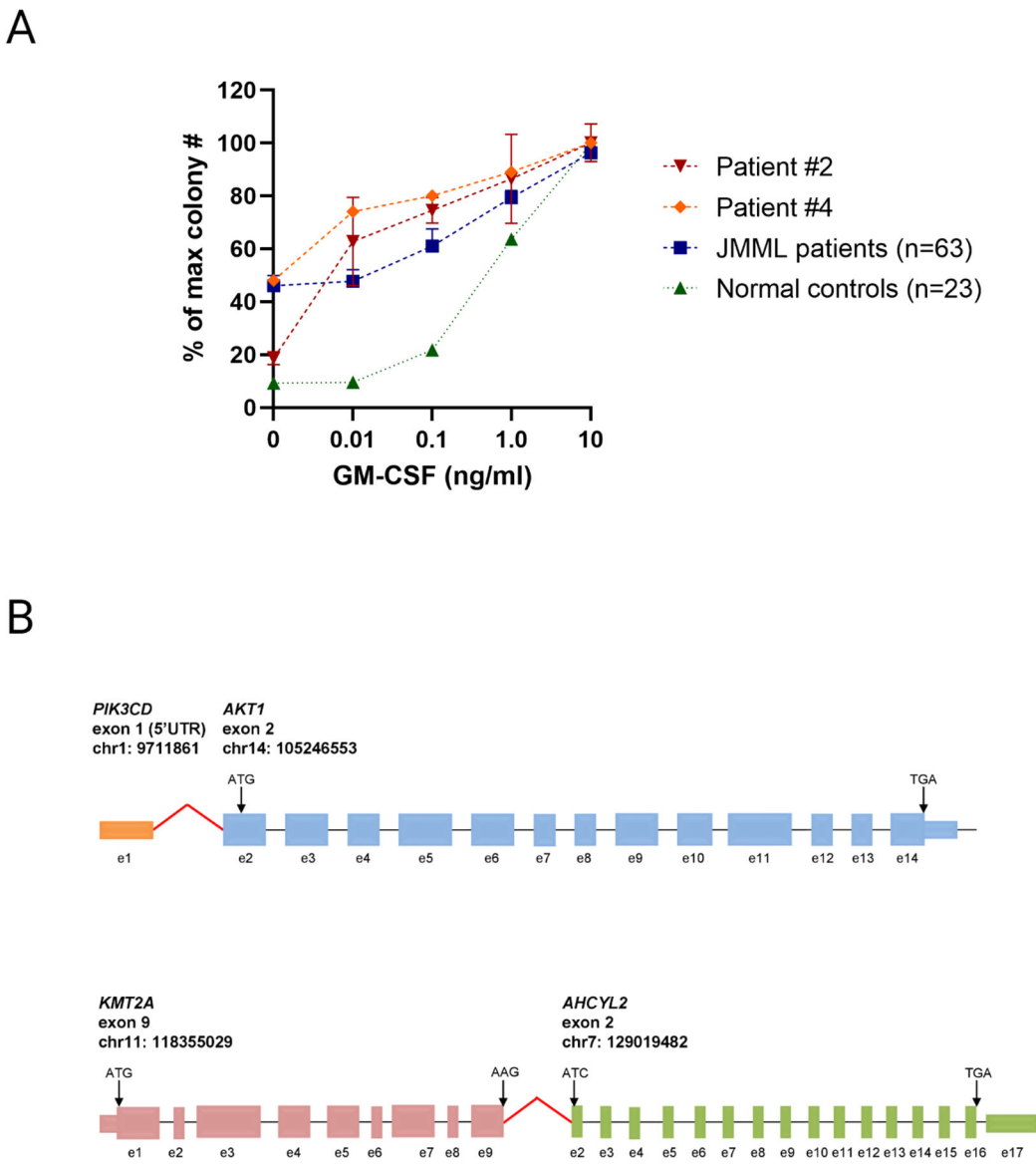

FIGURE 1.

Panel A: GM-CSF hypersensitivity assay of patient #2 and patient #4 in comparison to 23 normal healthy controls and 63 JMML patients that were previously performed in our laboratory. *Panel B:* Schematic illustration of fusions detected in patient 1.

TABLE 1

Clinical characteristics of patients described in this study.

Patient ID	Age at initial diagnosis (years)	Sex	First malignancy	Molecular alterations at diagnosis of first malignancy	Treatment first malignancy	Latency period from end of therapy of initial malignancy to diagnosis of t-MN (months)	Molecular alteration at diagnosis of t-MN	Karyotype at diagnosis of t-MN	Treatment t-MN	Outcome	Follow-Up from diagnosis of t-MN (months)
1	1.7	F	Hepatoblastoma	Not perfomed.	SIOPEL-4 Protocol	4	*KMT2A/AHCYL2* fusion; *PIK3CD/AKT1* fusion; *NRAS* p.Q61K (43%); *TP53* p.E286K (47%); *MLL2* p.P511fs (10%), *MYST4* p.D1664A (23%)	Complex	Cytarabine, Fludarabine, Hydroxyurea, 6-MP, HCT	Deceased	4
2	1.8	M	Pre B-ALL	Normal FISH & cytogenetics	AALL0932	3	*NF1* p.T676fs (56%); *NRAS* p.G13R (17%)	Monosomy 7	AAML1031 + HCT	Alive	54
3	12	M	Pre B-ALL	CDKN2A deletion	AALL1131	24	*CBL* p.R420G (95%); *RUNX1* p.L98Sfs (48%); *NRAS* p.G12D (41%); and *NRAS* p.G12V (6%)	Monosomy 7	AAML1031, Azacitidine, Decitabine Gemtuzumab (Refused HCT)	Deceased	19
4	3	M	Pre B-ALL	Trisomy 4 and 10.	AALL0232	36	*NRAS* p.G12A (Sanger)	Monosomy 7	Mitoxantron Cytarabine HCT	Alive	168
5	3	N/A	Atypical teratoid rhabdoid tumor	*SMARCB1* p.P383R fs*100 and heterozygous deletion of whole *INI1/SMARCB1* gene	DFCI ATRT Protocol	8	*KRAS* p.G12D (38%)	t(1;10) and t(4;6)	Cytarabine, Fludarabine, Azacitidine, Venetoclax, HCT	Alive	6

TABLE 2

Clinical characteristics of previously reported cases.

Patient ID	Age at initial diagnosis (years)	Sex	First malignancy	Treatment first malignancy	Latent period (months)	Molecular alteration at diagnosis of t-MN	Karyotype at diagnosis of t-MN	Treatment t-MN	Outcome	Follow-Up (months)	Ref
6	2.5	F	ALL	Methotrexate Vincristine Prednisone 6-MP Radiation	10	n/a	Abnormal	n/a	n/a	n/a	[22]
7	2.9	M	ALL	Vincristine Cyclophosphamide Methotrexate/6-MP Cranial Radiation	6	n/a	Monosomy 7	Hydroxyurea Etoposide Thioguanine Busulfan	Deceased	4	[25]
8	10	F	T-cell lymphoma	Cyclophosphamide Methotrexate Cytosine Arabinoside Vincristine Doxorubicin	17	n/a	46,XX, t(9;11) (p22;q23)	Cytosine Arabinoside, Daunorubicin Etoposide	CR		[23]
9	7	M	ALL	Vincristine Prednisone L-asparaginase Pirarubicin Cyclophosphamide Cytarabine Methotrexate/6-MP	21	n/a	46,XY, t(11;16) (q23;p13)	n/a	n/a		[24]
10	8.6	M	B-ALL	Vincristine L-asparaginase Daunorubicin Prednisone Etoposide Cytarabine	23	n/a	46,XY, t(11;17) (q23;q25,der(7), t(7;?)(q22;?)	n/a	Deceased	1	[26]