Secondary Precursor T-Cell Lymphoblastic Lymphoma Following Precursor B-cell Acute Lymphoblastic Leukemia: A Case Report and Review of the Literature

Jenny L. Smith[1], Albert Kheradpour[2], Craig W. Zuppan[1], Jun Wang[1], Rhett P. Ketterling[3] and Edward H. Rowsell[1,*]

[1]Departments of Pathology and Laboratory Medicine, and [2]Pediatric Hematology and Oncology, Loma Linda University Medical Center, Loma Linda, CA 92354, USA; [3]Department of Pathology and Laboratory Medicine, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

Abstract: Although relapse of lymphoma/leukemia is not uncommon, sequential development of a second lymphoma/leukemia of a different cell lineage is rare. We report the case of a 3-year-old girl who initially presented with precursor B-cell acute lymphoblastic leukemia (B-ALL), characterized by a cryptic t(12;21) with associated ETV6/RUNX1 fusion, an 11q (MLL) deletion, and a balanced inv(2)(q31q37). She was successfully treated but five years later developedthymicprecursor T-cell lymphoblastic lymphoma (T-LBL) expressing a completely different phenotypic profile. Fluorescence in situ hybridization testing identified a MLL rearrangement but indicated no ETV6/RUNX1 fusion. Although the marrow was uninvolved, aspirates evaluated by chromosome studies revealed the same inv(2q), suggesting a constitutional abnormality distinct from the somatic alterations associated with her B-ALL and T-LBL. This raisesthe possibilityof a potential tumor suppressor gene or proto-oncogene residing in the region of the inversion breakpoints which could contribute to predisposition to the development of lymphoblastic leukemias/lymphomas. While secondary leukemia may emerge as a therapy-related process and the presence of an MLL rearrangement in the T-LBL represents an interesting abnormality in this regard,athymicpresentation would be exceedingly unusual. To our knowledge, this is the first reported case of B-ALL followed by an apparently genetically unrelatedT-LBL.

Keywords: Secondary malignancy, acute lymphoblastic leukemia, acute lymphoblastic lymphoma, pediatrics, lineage difference.

INTRODUCTION

Although relapse of acute leukemia is not infrequent, second malignancies after childhood acute leukemia are uncommon occurrences. The most frequent secondary malignancy is acute myeloid leukemia (AML). Secondary acute lymphoblastic leukemia/lymphoma (sALL) is distinctly uncommon and studies on etiology and treatment are limited. Herein, we report a case of precursor B-cell acute lymphoblastic leukemia in a 3-year-old girl followed 5 years later by a precursor T-cell acute lymphoblastic lymphoma (T-LBL), and discuss existing theories on the etiology of sALL.

CASE REPORT

A 3-year-old girl presented with headache, intermittent fever for several weeks, rhinorrhea and pharyngitis with tachypnea and subsequent tachycardia. A complete blood count demonstrated pancytopenia with 12% circulating blasts, hemoglobin 3.0 g/L, platelets 3.0 x 10^9/L, and white cell count 2.58 x 10^9/L. Her lactate dehydrogenase was elevated at 499 U/L. Chest radiographs revealed atelectasis but no other intrathoracic abnormalities. A bone marrow biopsy and aspiration demonstrated blasts in the marrow similar to those seen in the peripheral blood. With supporting ancillary tests (see Pathologic and Genetic Findings) a diagnosis of precursor B-cell acute lymphoblastic leukemia (B-ALL) was made, and she was started on COG protocol AALL0331. After finishing the induction phase of therapy and starting consolidation, repeat bone marrow examinations were negative for malignancy. Complications of her hospital course included a reversible posterior leukoencephalopathy with new-onset seizure activity, bilateral intraretinal hemorrhages, infection at the site of her bone marrow biopsy, and hypertension. A complete and stable remission of her leukemia was obtained with therapy.

Five years later, she presented to the emergency department complaining of left shoulder pain. On admission, a complete blood count showed hemoglobin of 13.7 g/L, platelets 396 x 10^9/L, and white cell count 9.01 x 10^9/L. Her lactate dehydrogenase was again elevated, at 447 U/L. A chest x-ray demonstrated a large left-sided pleural effusion with complete loss of visualization of the left heart border. CT scan of the chest revealed a new 11 x 10 x 9 cm anterior

*Address correspondence to this author at the Department of Pathology and Laboratory Medicine, Loma Linda University Medical Center, Room 2516, 11234 Anderson Street, Loma Linda, CA 92354, USA; Tel: (909) 558-6001; Fax: (909) 558-4113; E-mail: erowsell@llu.edu

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mediastinal mass (Figure **1**). Biopsies of this mass showed findings consistent with precursor T-cell lymphoblastic lymphoma. A peripheral blood smear and a bone marrow biopsy to evaluate for systemic involvement were negative. She was treated per protocol AALL0434.

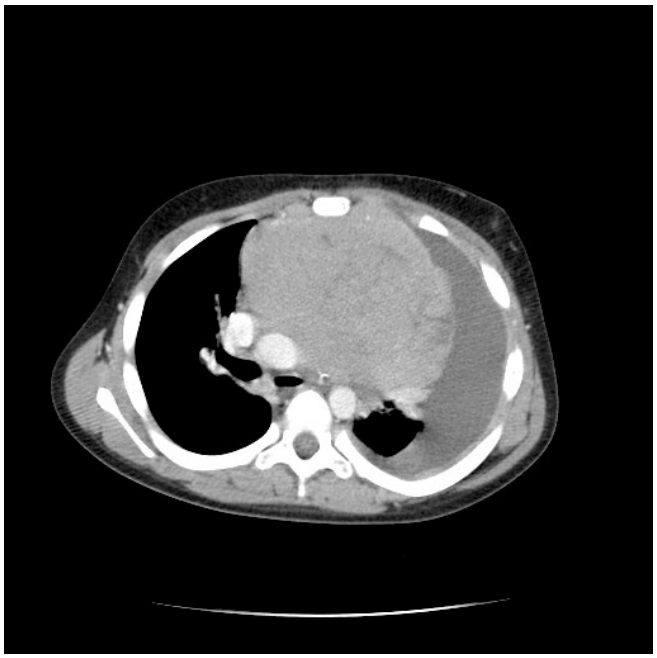

Figure 1: CT scan showing large mediastinal mass with accompanying left pleural effusion at the time of second presentation.

As of this writing, four years after completing COG protocol AALL0331 chemotherapy for B-ALL, she remains in remission. One year after initiating protocol AALL0434 for T-LBL, she is on maintenance chemotherapy and clinically stable. The only sequelae she has encountered are anxiety and memory deficits, possibly related to neuropsychological issues associated with her medical diagnosis.

PATHOLOGIC AND GENETIC FINDINGS

Although the marrow aspirate smear was extremely pauci-cellular due to a dry tap and marked hemodilution, the cells present were nearly all lymphoblasts (Figure **2**). The hematoxlin and eosin-stained bone marrow sections from the first biopsy at age 3 demonstrated a hyperplastic marrow almost entirely replaced by sheets of lymphoblasts with high nuclear to cytoplasmic ratios and scant basophilic, agranular cytoplasm. By flow cytometry, the blastic population expressed CD10, CD19, CD20, CD34, CD45, CD79a, and TdT but did not express CD22, CD3, CD4, CD7, or CD8 (Table **1**). There was also aberrant myeloid antigen CD13 expression. The morphologic and immunophenotypic findings were most consistent with B-ALL. Bone marrow chromosome studies demonstrated an inv (2) (q31q37) in all 20 metaphases and a deletion (11q) in 9 metaphases. FISH studies confirmed the 11q deletion by demonstrating loss of the MLL gene (at 11q23) and revealed ETV6/RUNX1 fusion, indicating a “cryptic” t (12;21) (p13;q22).

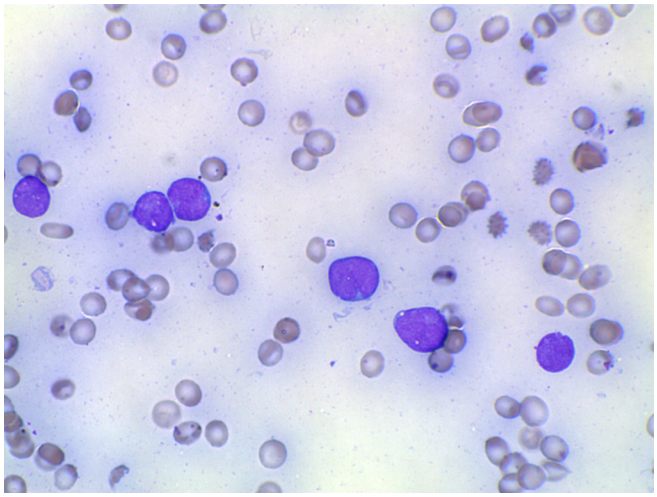

Figure 2: Precursor B-cell acute lymphoblastic leukemia: initial marrow aspirate (Wrigth-Giemsa stain, original magnification x 600).

Histological examination of the subsequent mediastinal mass biopsy at age 8 showed a homogeneous population of small blastic lymphoid cells with slight nuclear irregularity, small nucleoli, and moderate mitotic activity (Figure **3A**). By flow

Table 1: Summary of Tumor Phenotype

	Flow Cytometric Markers (Blast Population)	
	Positive	**Negative**
Initial B-ALL[a]	CD10, CD20, CD34, CD45, CD79a, TdT[c], CD13	CD3, CD4, CD7, CD8
Subsequent T-LBL[b]	CD3, CD4, CD7, CD8, CD34, CD45, CD10	CD19, CD20, CD13

[a]B-cell acute lymphoblastic leukemia.
[b]T-cell acute lymphoblastic lymphoma.
[c]Terminal deoxynucleotidyltransferase.

Table 2: Summary of Somatic Genetic Alterations

	MLL[c]	ETV6/RUNX1[d] fusion
Initial B-ALL[a]	Deletion	Present
Subsequent T-LBL[b]	Rearrangement with unknown fusion partner	Not identified

[a]B-cell acute lymphoblastic leukemia.
[b]T-cell acute lymphoblastic lymphoma.
[c]Mixed lineage leukemia.
[d]Ets variant 6; runt-related transcription factor 1.

cytometry, these tumor cells expressed CD7, CD8, CD3, CD4, CD10, CD34, and CD45; but did not express CD19, CD20, or CD13 (Table **2**). Immunohistochemical stains on tissue sections demonstrated positivity for TdT and CD3 (Figures **3B** and **3C**) but negativity for PAX5, CD20, CD15, and CD30. These morphologic and immunophenotypic findings were most compatible with T-LBL.

While the bone marrow was morphologically uninvolved by the T-LBL, cytogenetic studies were performed on bilateral bone marrow specimens and demonstrated the inv (2)(q31q37) in all 20 metaphases from each bone marrow chromosome study. FISH was performed on the T-LBL mediastinal mass specimen and demonstrated an MLL rearrangement in 41% of the nuclei without evidence of translocation to any of

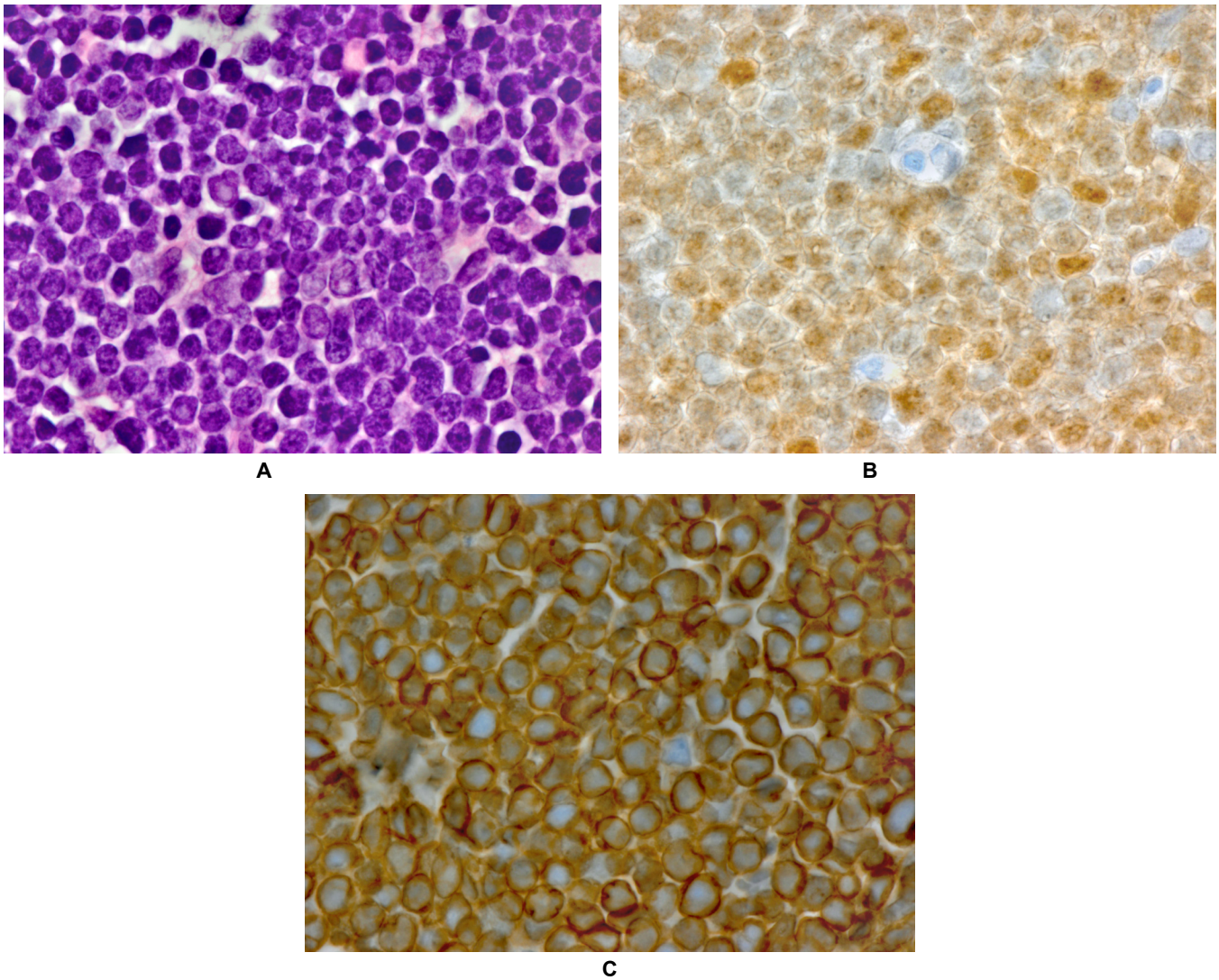

Figure 3: Precursor T-cell acute lymphoblastic leukemia/lymphoma: **A**. Mediastinal mass (H&E, original magnification x200). **B**. Lymphoblasts expressing terminal. deoxynucleotidyltransferase on immunohistochemistry (original magnification x200). **C**. Demonstration of CD3 positivity of the blasts (original magnification x200).

the most common partner gene loci (i.e. AFF1, MLLT3, MLLT4, MLLT10, ELL, or MLLT1). In addition, FISH testing for ETV6 and RUNX1 was performed and was normal.

Array-comparative genomic hybridization analysis (aCGH) was performed on peripheral blood and did not identify any gains or losses at 2q31 or 2q37 at the resolution evaluated with the SNP array (approximately 30-50 kilobases). The aCGH analysis did identify an interstitial deletion of 622 oligonucleotide probes at 16p13.3, spanning roughly 188 kilobases, involving the RBFOX1 gene (too small to be confirmed by FISH). However, maternal aCGH revealed the same interstitial deletion, indicating a maternal inheritance to this microdeletion. Of note, the patient's mother has no known history of malignancy. Further genetic evaluation of the proband included sequence analysis for dyskeratosiscongenita, a condition that may predispose affected individuals to bone marrow dysfunction, myelodysplasia, or leukemia, which was negative for mutations in the DKC1, TINF2, TERC, NHP2, NOP10, and TERT genes. In addition, DNA sequence analysis of the TP53 gene (exons 2 through 11) did not identify mutations or sequence variants.

DISCUSSION

B-ALL is one of the most common childhood malignancies, accounting for nearly one quarter of all malignancies in patients under 15 years of age [1]. Identification and description of genetic abnormalities in acute leukemias has helped to elucidate the pathophysiology and guide risk stratification and treatment strategies [2]. Multiple prognostic genetic subgroups have been delineated in B-ALL with the most common recurrent translocation, t(12;21)(p13;q22) occurring in approximately 20-25% of pediatric patients [3]. Translocations of the mixed-lineage leukemia (MLL) gene, residing at 11q23, are particularly frequent in infant leukemia, accounting for 80% of ALL and 60% of AML cases [3, 4]. MLL translocations occur in all acute leukemia subtypes (AML, B-ALL and T-ALL) in the pediatric age group, and occur with a similar frequency of approximately 5-10%. MLL translocations are generally thought to indicate a poor prognosis. However, over 100 different fusion sites have been described, and studies now suggest that the specific fusion partner gene is of great importance [4, 5]. The presence of additional chromosomal abnormalities also portends a worse prognosis than isolated MLL translocations [6].

In our patient, the initial B-ALL clone occurred at age 3 and was characterized by ETV6/RUNX1 fusion and an 11q deletion (MLL deletion by FISH). However, the subsequent T-LBL clone occurring at age 8 was characterized by an MLL rearrangement and a lack of ETV6/RUNX1 fusion. An apparent constitutional chromosome 2 inversion at q31 and q37 was identified at both time points. Array CGH indicated this inv (2) (q31q37) did not result in any gain or loss of DNA at the level of resolution of approximately 30-50 kilobases. However, a possible tumor suppressor gene or proto-oncogene residing in the region of the inversion is possible which may predispose this child to develop acute lymphoblastic leukemia. Of interest, mutations of 2q31 (HOXD4) have been previously described by van Scherpenzeel Thim, *et al*., in two cases of acute lymphoblastic leukemia; however further studies of this region are necessary before drawing any conclusions [7].

This child also had a constitutional interstitial deletion of 16p13.3 in what is thought to be a non-coding region of the RBFOX1 gene. The RBFOX1 gene regulates tissue-specific splicing by binding mRNA precursors. While point mutations within this gene have been associated with epilepsy, intellectual disability, and autism [8, 9], this region on chromosome 16p13.3 is a known region of polymorphic duplication and deletion without apparent phenotypic consequence. In addition, since the mother shares the same microdeletion and has no known malignancies or other obvious abnormalities, the maternally inherited 16p13.3 microdeletion likely representsa familial variation without significant impact on phenotype. However, there still exists the possibility that this is a deleterious deletion with minimal expression in the mother or that the deletion is not deleterious by itself, but rather a corresponding mutation in the allele on the second chromosome 16 within the deletion region in the proband could inactivate a gene of interest, thus unmasking an "autosomal recessive" type scenario and generating a complete loss of a tumor suppressor or proto-oncogene within this region.

Although secondary malignancies are well-recognized following treatment of a primary neoplasm, secondary malignancies following treatment of childhood acute lymphoblastic leukemia are uncommon. Among second malignancies, secondary AML predominates in pediatric patients, with myelodysplastic syndrome and non-meningioma brain tumors also occurring with some frequency [10]. Secondary acute lymphoblastic leukemia/lymphoma

(sALL) is distinctly uncommon. Because of the rarity of sALL, little is known about its etiology but a number of pathogenic theories have been proposed.

One theory suggests that sALL actually represents relapse or persistence of a minor component in an initial biphenotypic/biclonal neoplasm, originating from a common progenitor cell, in which a minor subpopulation initially goes undetected [11]. Subsequently, this subpopulation may then emerge as an apparent "second malignancy". Theoretically, the residual tumor cells could possibly dedifferente, redifferentiate, and then clonally expand, manifesting as a new malignancy [12]. Still another potential mechanism of secondary malignancy is that of genetic damage from cytotoxic treatment protocols [10-11, 13-15].Genetic predisposition to the development of cancer or gene-environment interactions may contribute to true second malignancies [13].

The development of secondary ALL after treatment of acute leukemia may be misinterpreted as relapse by the unwary, and therefore under-reported. To clarify the true incidence of sALL, Zuna, *et al.*, analyzed children with clinically relapsed ALL following treatment with Berlin-Frankfurt-Munster-based protocols in four European centers. Of the 366 cases investigated, five cases (1.5% of those analyzed) showed no clonal relationship between the initial and recurrent disease, representing possible real secondary malignancy, either from a pre-leukemic progenitor cell or a novel pathway, rather than relapse. Two of those cases (0.5% of those analyzed) fulfilled their criteria of "pure sALL" (Table **3**) [11]. One case was that of a 5.1-year-old girl initially diagnosed with B-ALL. She relapsed once, then subsequently developed T-ALL with a new MLL rearrangement and fusion to MAML2 at 11q21. She had received radiation, etoposide, daunorubicin, and cyclophosphamide/ifosfamide prior to acquiring T-ALL. The second case was a 5.8-year-old boy first found to have B-ALL with aETV6/RUNX1 fusion. After treatment with etoposide, daunorubicin, and cyclophosphamide/ifosfamide, he developed T-ALL [10]. Our patient fulfilled Zuna's criteria for pure sALL since cytogenetic abnormalities and fusion genes differed from the first presentation to the second, and were accompanied by a clear immunophenotypic lineage switch.

Secondary neoplasms after treatment of an initial ALL are even more rare than sALL following other malignancies [13]. This finding may be at least partially due to the lower risk of treatment protocols for ALL compared to those used for other childhood cancers (e.g. small round blue cell tumors, sarcomas, Hodgkin's disease, etc) [14]. Treatment related AML (t-AML) has been reported not infrequently following treatment with radiation, epipodophyllotoxins, or topoisomerase II (topo-II) chemotherapies. A proportion of cases, especially those associated with topo-II chemotherapies, show balanced translocations involving the MLL gene at chromosome band 11q23 [15]. Similarly, MLL rearrangements have been reported in treatment-related ALL (t-ALL), but the incidence is far lower [16]. In our case, the patient did not receive radiation, epipodophyllotoxins, or topo-II chemotherapies after the initial B-ALL. Additionally, solid mediastinal lymphoblastic lymphoma has not been described as a treatment-related second malignancy to our knowledge. For these reasons, the thymicT-LBL in our patient appears relatively unlikely to be related to prior treatment, despite the presence of an MLL separation.

CONCLUSION

Secondary malignancy following precursor B-cell acute lymphoblastic lymphoma is rare. In our patient, the divergent immunophenotypes of the tumor cells in association with disparate genetic alterations argue for a true secondary malignancy, rather than relapse. Although there are two other case reports of T-ALL following treatment of B-ALL, these patients had received radiation and/or topo-II chemotherapy. To our knowledge, this patient represents the first reported case of solid (mediastinal) T-LBL following a B-ALL. The potential association with the constitutional inversion, inv(2)(q31q37), or less likely the interstitial microdeletion at 16p13.3, is intriguing and raises the consideration of a possible proto-oncogene or tumor suppressor gene at or near the inversion junctions or

Table 3: Criteria for Pure Secondary ALL[a], Proposed by Zuna *et al*.

No clonal relationship between diagnosis and recurrence (Ig/TCR[b], fusion genes at DNA level, cytogenetic marker).
-AND-
Significant immunophenotypic shift (i.e. lineage switch), OR significant cytogenetic shift, OR gain or loss of fusion gene

[a]Acute lymphoblastic leukemia.
[b]Immunoglobulin/T-cell receptor.

within the deletion region. Study of other patients with genetic abnormalities involving these chromosome locations is needed to clarify whether inversions or deletions in these regions are truly oncogenic.

DISCLOSURES

The authors have no conflicts of interest or funding to disclose.

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