Case Study

B-Lymphoblastic Leukemia/Lymphoma with CRLF2 Overexpression: A Case Study of Three Patients

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Abstract: B-lymphoblastic leukemia/lymphoma with CRLF2 overexpression belongs to the *BCR-ABL1*-like B- lymphoblastic leukemia / lymphoma group (*BCR-ABL1*-like B-ALL), also known as Philadelphia chromosome-like B-ALL, a recently recognized provisional entity in the latest edition of World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues (2016). Although lacking the classic t(9;22) *BCR-ABL1*, *BCR-ABL1*-like B-ALL shares a similar gene expression profile with *BCR-ABL1* translocation associated B-ALL. *BCR-ABL1*-like B-ALL often have translocations involving *CRLF2*, *EPOR*, or other tyrosine kinases. Due to prognostic importance and potential therapeutic differences, it is important to recognize and diagnose *BCR-ABL1*-like B-ALL in a timely manner. In this report, we describe a series of three adult patients with the diagnosis of B-ALL with CRLF2 overexpression and present all relevant clinical and pathologic findings.

Introduction

BCR-ABL1-like B-lymphoblastic leukemia/ lymphoma (*BCR-ABL1*-like B-ALL) is a subcategory of B-ALL and a new provisional entity in the 2016 edition of WHO. The frequency of *BCR-ABL1*-like B-ALL varies but increases with age from approximately 10% among children with standard-risk B-ALL up to approximately 27% among young adults with B-ALL or children with high-risk B-ALL [1, 2]. Clinical presentations of *BCR-ABL1*-like B-ALL are generally similar to those seen in patients with other types of B-ALL. *BCR-ABL1*-like B-ALL is characterized by a similar gene expression profile as BCR-ALB1 rearranged B-ALL, but lack the characteristic t(9;22). Unlike some other subcategories of B-ALL characterized by a single defining chromosomal aberrancy, *BCR-ABL1*-like B-ALL is defined by heterogeneous genetic alterations resulting in activation of tyrosine kinase signaling pathways [1–4]. About half of *BCR-ABL1*-like B-ALL cases demonstrate CRLF2 overexpression by way of *CRLF2* gene rearrangement [2, 5, 6]. *CRLF2*, or cytokine receptor-like factor 2, is located on chromosome Xp22.3 and Yp11.3, and along with the IL7R alpha (IL7RA) unit, forms the thymic stromal lymphopoietin receptor (TSLRP). Genomic rearrangements of *CRLF2* gene result in overexpression of CRLF2 itself leading to *JAK2*-mediated activation of STAT5, which promotes B-cell precursor proliferation and high rate of disease relapse [2]. These cases often show an interstitial deletion of the PAR1 gene family on Xp22.3 and Yp11.3, which juxtapose *CRLF2* to the promoter of P2RY8 gene. Alternatively, *CRLF2* translocation can occur with

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IGH. Aside from *CRLF2* rearrangements, translocations involving tyrosine kinases including *ABL1*, *ABL2*, *JAK2*, *PDGFRB*, and *CSF1R* have also been described [1, 2]. Many cases of *BCR-ABL1*-like B-ALL also show deletions in IKZF1 but without significant influence on overall survival [2]. Targeted sequencing studies have also found mutations in *JAK2* in *CRLF2* rearranged cases at as high as 50% [2]. From a therapeutic standpoint, the aberrancies associated with ABL-class tyrosine kinase and *JAK* have opened the door for tyrosine kinase inhibitors and *JAK* inhibitors as alternative treatment since many of these patients are resistant to induction chemotherapy.

The genetic heterogeneity of *BCR-ABL1*-like B-ALL poses challenges for diagnosis. Initially, *BCR-ABL1*-like B-ALL was described within pediatric patients by two different research groups using different methodologies for gene expression profiling [7, 8]. However, there was imperfect concordance using the criteria from each of these groups to classify cases as *BCR-ABL1*-like B-ALL [9]. In addition, the genetic aberrancies may have different breakpoints, different fusion transcripts, or different fusion partners, further complicating accurate classification [10]. Despite these challenges, identifying *BCR-ABL1*-like B-ALL patients is clinically significant for prognosis and potentially for therapy since retrospective studies have demonstrated significantly worse outcomes for *BCR-ABL1*-like B-ALL patients compared with other B-ALL subtypes in adults [2, 6]. Within the last few years, developments in multiparameter flow cytometry (MPFC) testing, targeted fluorescence in-situ hybridization (FISH) assays, and next generation sequencing (NGS) assays have improved the speed and accuracy for triage and diagnosis of these patients. MPFC is a relatively fast and simple way to detect cell surface CRLF2 expression with a high concordance rate of *CRLF2* rearrangement [11]. At our institution, this approach has been in clinical practice since 2017 as it facilitates confirmatory FISH and molecular testing for prognosis and therapy. Currently, there are no clear guidelines for the screening of *BCR-ABL1*-like B-ALL. As further research better characterizes the prognostic and therapeutic implications of specific genetic aberrancies within the disease, clinical strategies to capitalize on targetable aberrancies will become more apparent.

In this case study, we describe three adult patients diagnosed with B-ALL with CRLF2 overexpression from our institution, including all relevant findings related to clinical, pathology, therapy, and survival outcomes. The purpose is to better understand the disease process from a case-based format and to create discussion and hopefully implement change in everyday practice.

Case Report

Case 1

A 26-year-old female with no prior significant past medical history presented with abdominal pain, severe pancytopenia, and 49% blasts on peripheral blood smear in December, 2016. Her bone marrow biopsy showed 93% blasts by morphology, and 97% B-lineage lymphoblasts by flow cytometry that expressed CD10, CD19, partial weak CD20, CD22, CD34, and TdT. CRLF2 expression by flow cytometry was not available at the time of the initial diagnosis. A targeted B-ALL FISH panel demonstrated a 14q32 (*IGH*) rearrangement without evidence of *MYC*, *BCR-ABL1*, *MLL*, or *TCF3* rearrangements. Conventional karyotype showed normal 46,XX. Based on the lack of BCR-ABL translocation and a 14q32 (*IGH*) rearrangement, there was suspicion for *BCR-ABL1*-like B-ALL. Subsequently, a comprehensive NGS panel was performed at an outside institution and demonstrated an *IgH-CRLF2* rearrangement. Additional findings included mutation in *JAK2* T875N in a subclone, loss of CDKN2A/B, and loss of RB1 exons 18-27. Based on these findings, a diagnosis of *BCR-ABL1*-like B-ALL was made. Given the concurrent *JAK2* mutation, the possibility of a *JAK* inhibitor was suggested. In addition to bone marrow involvement, there was also CNS involvement.

Initial treatment included CALGB10403 protocol

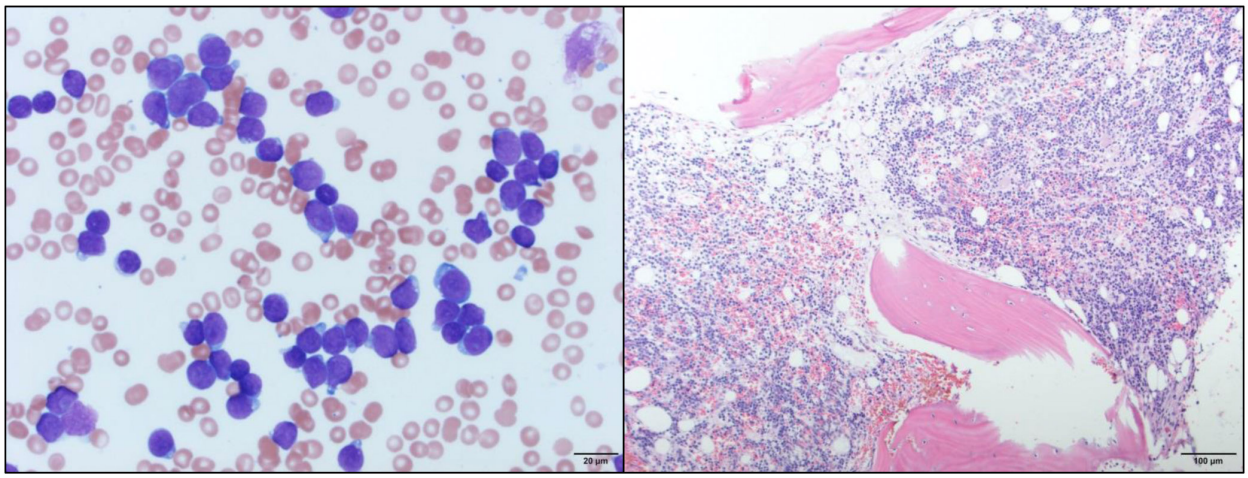

Figure 1: Bone marrow aspirate and core biopsy from Patient #2. The morphologic features of *BCR-ABL1*-like B-ALL are not unique and are similar to other B-ALL. Aspirate at 500x magnification (left panel) and core biopsy at 100x magnification (right panel).

with intrathecal methotrexate. Her course of therapy was complicated by uncontrolled hyperglycemia requiring insulin, pyelonephritis, nausea/vomiting, near syncope, hyperbilirubinemia, and elevated liver function tests. Pathologic response was assessed by a repeat bone marrow biopsy on Day 28, and showed flow cytometric evidence of low-level residual disease (0.5% B-lymphoblasts). The targeted B-ALL FISH panel was repeated, and the 14q32 (*IGH*) rearrangement was no longer detectable. Repeat bone marrow biopsies following course two of chemotherapy and while on interim maintenance therapy demonstrated no morphologic or flow cytometric evidence of residual/recurrent B-ALL each time. Repeat FISH panels also showed no detectable rearrangements. Serial assessments of CSF by morphology and flow cytometry demonstrated persistent involvement on Day 8 of CALGB10403 protocol, but were subsequently negative. The patient then underwent an allogeneic haploidentical peripheral blood stem cell transplant during her first complete remission after three cycles of interim maintenance therapy. Again, her post-transplant course was complicated by MICU admission for possible sepsis, *C. difficile* infection, mucositis, recurrent epistaxis, pneumonia versus fluid overload, pericardial effusion, acute respiratory failure, and hemorrhagic cystitis. Repeat bone marrow biopsy at Day 89 of post-transplant in September, 2017 demonstrated no morphologic or flow cytometric evidence of residual/recurrent B-ALL. A repeat ALL FISH panel showed no detectable rearrangements. Intrathecal methotrexate was continued for 3 additional months. To date, the patient is disease free.

Case 2

The patient is a 30-year-old male with a prior diagnosis of B-ALL from 2012 after presenting with fevers, easy bleeding, and pancytopenia. His diagnostic bone marrow biopsy demonstrated 94% blasts by morphology, 90% B-lymphoblasts by flow cytometry, which also expressed CD10, CD19, weak CD22, CD34, and TdT, but did not express surface light chains or CD20. CRLF2 by flow cytometry was not available at the time. There was no CSF involvement.

Initial treatment included CALGB10403 protocol with induction and four cycles of intensification chemotherapy. A Day 21 bone marrow biopsy showed no definitive evidence of residual disease. The patient was started on maintenance therapy for

the next three years. Subsequent bone marrow biopsies from 2013-14 all showed no evidence of residual disease. In early 2015, a surveillance bone marrow biopsy demonstrated 0.1% B-lymphoblatsts by flow cytometry suspicious for recurrent disease. The next four bone marrow biopsies in 2015 showed the same B-lymphoblast population ranging from 0.2-2%. In the summer of 2015, the B-lymphoblast population started to show aberrant CD33 expression by flow cytometry. Approximately two months after the maintenance therapy, a surveillance bone marrow biopsy in early 2016 demonstrated definitive disease relapse with 87% lymphoblasts by morphology, 20% by flow cytometry. The patient was then re-induced by COG-ALL 1331 protocol, and at the end of re-induction, there was no evidence of residual disease. The decision at that time was to move to transplant and the patient subsequently underwent a match unrelated donor (MUD) transplant. At Day 45 post-transplant, a bone marrow biopsy showed no evidence of residual disease; however, at Day 100 post-transplant, there was 0.1% B-lymphoblasts by flow cytometry. He subsequently underwent re-induction again with clofarabine and dexamethasone in October of 2016. A repeat biopsy at that time showed full relapse with significant increase in blasts [Figure 1]. By this time, CRLF2 expression by flow cytometry had been validated for clinical lab testing and was positive in this patient [Figure 2]. Due to the poor treatment response, failed transplant over the course of three years, and the positive CRLF2 by flow cytometry, further testing for *BCR-ABL1*-like B-ALL was performed. A NGS panel showed the presence of an *IGH-CRLF2* rearrangement, *CDKN2A/B* p14ARF loss of exon 1, *CDKN2b* loss, *IKZF1* C119fs*2 mutation, and *PAX5* I301T mutation. This confirmed the diagnosis of *BCR-ABL1*-like B-ALL. The patient was then started on blinatumomab, ruxolitinib, prednisone, and inotuzumab for relapsed/refractory B-ALL. A follow up bone marrow biopsy in early 2017 showed complete remission by morphology and flow cytometry. However, just two months later, a small population of atypical B-lymphoblasts was again detected by flow cytometry on a bone marrow biopsy. At this time, he also developed evidence of extramedullary disease involving the parotid gland, periorbital area, and spleen. A biopsy of the parotid gland showed involvement by B-ALL. Due to repeated therapy failure, he was then referred for CAR-T cell therapy as part of a research trial. The new therapy initially appeared successful since multiple bone marrow biopsies demonstrated no evidence of disease over the next six months. However, a bone marrow karyotype showed new abnormality of del(7)(q22q32) present in 15% of cells, as well as new mutations including *TP53* and *DNMT1*. A follow up bone marrow biopsy in October of 2017 showed significant disease relapse with 88% blasts, a new *KDM6A* mutation, an 11q23 deletion, and a 14q32 rearrangement by FISH analysis. He was treated with hyperCVAD-B and reinduced with gemtuzumab (due to aberrant CD33 expression of the lymphoblasts) and ATRA. A post induction bone marrow biopsy showed no improvement in his disease process. His treatment course was also complicated by furunculosis, possible pulmonary aspergillosis, bilateral pleural effusions, infectious enterocolitis with ascites, anasarca, subdural hematoma, encephalopathy, and severe pancytopenia with transfusion dependence. A pleural fluid demonstrated involvement by B-ALL. By the end of 2017, the decision was made to transition to comfort care and the patient passed away shortly thereafter. The suspected cause of death was infection; however, no additional testing or autopsy was performed. One last bone marrow biopsy performed approximately 10 days before the patient died showed high level of residual B-ALL.

Case 3

The patient is a 40-year-old male initially diagnosed with B-ALL at age 38 from an outside hospital with 80.8% blasts by morphology, 84% B-lymphoblasts by flow cytometry. CRLF2 expression by flow cytometry was not available at this time. At initial diagnosis, a karyotype was normal and there was no

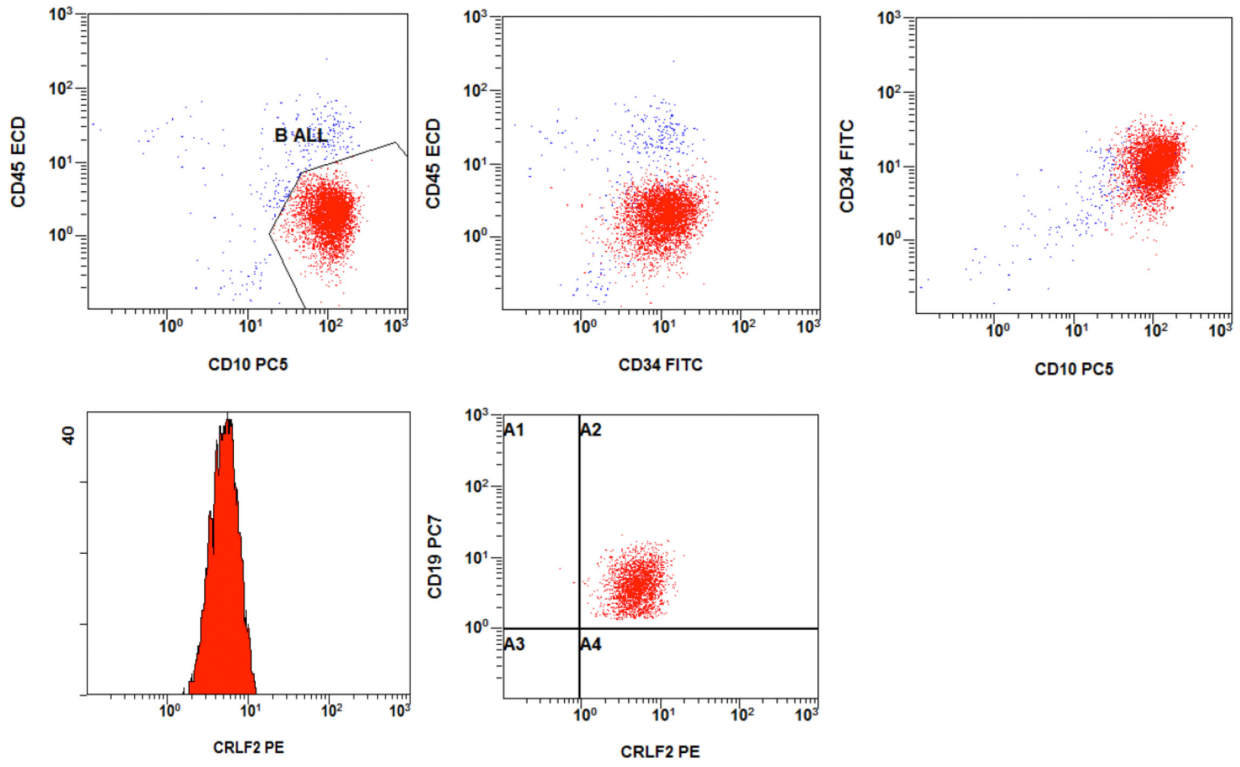

Figure 2: Flow cytometric analysis for CRLF2 expression from Patient #2. A B-lineage blast population (red) is shown with co-expressions of CD10, dim to negative CD45, CD34, CD19, and surface CRLF2.

evidence of recurrent genetic aberrancies including *CEP9*, *BCR-ABL*, *MLL*, chromosome 4, chromosome 10, and *TEL/AML1*.

He was initially treated with four cycles of hyper-CVAD followed by maintenance therapy with POMP (purinethol, oncovin, methotrexate, prednisone). He responded to therapy with no definitive morphologic or flow cytometric evidence of residual disease. However, 16 months after the diagnosis, he presented to the hospital with fever and increased white blood cell count which prompted a bone marrow evaluation. The biopsy showed recurrence of disease with >95% blasts. Flow cytometry analysis of the bone marrow aspirate showed similar findings as well as co-expression of CRLF2. With high suspicion for *BCR-ABL1*-like B-ALL, targeted interphase FISH was performed and demonstrated *CRLF2* rearrangement involving deletion of the *CRLF2* proximal region and gain of the derivative chromosome X resulting in *CRLF2-P2PY8* fusion, which was present in 94% of the cells scored. These findings are consistent with the diagnosis of *BCR-ABL1*-like B-ALL. At this time the patient was transferred to our institution, and he was initially treated with weekly vincristine. A follow-up bone marrow biopsy demonstrated no evidence of disease; however, his treatment course was complicated by multiple infections, including bacterial sepsis, *Candida glabrata* fungemia with bilateral eye involvement, tumor lysis syndrome, hemophagocytic lymphohistiocytosis, upper gastrointestinal bleeding, encephalopathy, acute renal failure, and myopathy of critical illness. He was transitioned to comfort care following worsening encephalopathy and passed away shortly thereafter. An autopsy was performed and showed extensive leukemic involvement of most internal organs in-

cluding heart, lungs, liver, spleen, and kidneys. In addition, the leptomeninges demonstrated a diffuse leukemic infiltrate forming bilateral subdural pseudomembranes. The total time of survival from initial diagnosis was 19 months.

Discussion

BCR-ABL1-like B-ALL is a genetically complex and clinically high-risk disease characterized by a gene expression profile similar to *BCR-ABL1* rearranged B-ALL but lacking the translocation. Instead, most cases carry a *CRLF2* rearrangement along with mutations in *JAK*. To date, the diagnostic process is still complex and challenging for routine practice. Here we present three cases of B-ALL with CRLF2 overexpression along with their clinicopathologic features and outcomes.

A few points of emphasis are worthy of discussion. 1) As demonstrated by these cases, the clinical course and prognosis is rather bleak. Despite numerous attempts of conventional and novel therapy, including *JAK* inhibitor, and transplants, two of the three patients passed away in less than five years from initial diagnosis. The follow up on the first patient is relatively short therefore whether the treatment has ultimately been successful is still unclear. Patients #2 and #3 showed us that the disease has a high relapse rate in a short amount of time, and that lack of response to therapy is common. 2) CRLF2 expression analysis by flow cytometry is a useful tool in the triage of all B-ALL patients. Given that the prevalence of *BCR-ABL1*-like B-ALL, especially in adults, is upwards of 20-30%, there is utility in screening all B-ALL patients for CRLF2 expression. This is well illustrated in Patient #2. Prior to the clinical availability of CRLF2 by flow cytometry, the patient was presumed to have a diagnosis of B-ALL, NOS and managed over two years with repeat therapy and transplant failures. When CRLF2 flow cytometry testing became available, the patient was accurately identified to be CRLF2 positive and subsequently tested and found to carry a *CRLF2* rearrangement. This changed his treatment strategy and risk stratification. Although subsequent therapy also failed and the patient did not survive much longer, it is not unreasonable to speculate that if he had been identified with *BCR-ABL1*-like B-ALL at the time of initial diagnosis, his clinical course might have been different. 3) Finally, based on the examples set by these three patients, a consensus guideline for the triage and identification of *BCR-ABL1*-like B-ALL patients should be established. Similar to the experience published by Konoplev *et al*. at MD Anderson [11], we agree that the best approach for the screening of *BCR-ABL1*-like B-ALL patients at this time is by flow cytometric analysis of CRLF2. If CRLF2 expression is positive, the patient is presumed to have a diagnosis of *BCR-ABL1*-like B-ALL, which can be confirmed by targeted interphase FISH, and further molecular testing for *JAK* mutation is warranted. Unfortunately, this approach only covers about half of the *BCR-ABL1*-like B-ALL cases, and there is currently no rapid screening method to identify cases with *EPOR* or other tyrosine kinase translocations.

In this case series, we have described the clinical course of three adult patients with B-ALL with CRLF2 overexpression, illustrated the aggressive nature of the disease, and demonstrated the need for consensus guidelines for the identification of these high-risk patients. With earlier identification of *BCR-ABL1*-like B-ALL, alternative treatments such as tyrosine kinase and *JAK* inhibitors may prove to be beneficial and improve outcome; however, further research is required for better understanding.

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