P2RY8-CRLF2 Fusion–Positive Acute Myeloid Leukemia With Myelodysplasia-Related Changes: Response to Novel Therapy

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INTRODUCTION

IGH–cytokine receptor–like factor 2 (*CRLF2*) rearrangement is the most common mechanism of overexpression of CRLF2 in precursor B-cell acute lymphoblastic leukemia (B-ALL); however, *P2RY8-CRLF2* rearrangement or alternative alterations leading to CRLF2 overexpression have also been observed, which are associated with increased relapse rate and progression.[1] Although many myeloid neoplasms, particularly acute myeloid leukemia (AML), are associated with various recurrent genetic rearrangements and fusions,[2] alterations leading to CRLF2 overexpression are exceedingly rare in AML. To date, no patient with myeloid neoplasm with *CRLF2* translocation has been reported. One patient with myelodysplastic syndrome with excess blasts-1 (MDS-EB1) with CRLF2 overexpression via multiple copies of an isodicentric Y chromosome, without evidence of *CRLF2* rearrangement, has been reported.[3] To our knowledge, we report the first patient with AML arising from an MDS, with a *P2RY8-CRLF2* fusion, which responded to a myeloid regimen in a clinical trial of hypomethylating agent and immune checkpoint blockade combination therapy. The study was performed in accordance with the Helsinki declaration. Patient consent has been obtained, and the study has been approved by the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board under protocol 12-245.

CASE REPORT

A 78-year-old man had an initial diagnosis of MDS-EB1 for 1 year, with bone marrow showing 8% blasts by aspirate differential count and 5%-9% blasts by CD34 immunostain (Appendix Fig A1A). He was monitored without any treatment or transfusions before presenting to MSKCC with worsening pancytopenia, including severe neutropenia. Bone marrow examination showed approximately 40% blasts that were medium in size with a rim of vacuolated cytoplasm, dispersed chromatin, and inconspicuous nucleoli (Fig 1A), consistent with progression to AML. Flow cytometry confirmed the presence of an expanded blast population with predominantly abnormal myeloid immunophenotype, but a minute subset of blasts dimly expressed CD19 (Fig 1B). Despite this, the patient is best classified as having AML with myelodysplasia-related changes (MRC) based on the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemias.[2]

COMPREHENSIVE GENOMIC/TRANSCRIPTOMIC PROFILING

Fluorescence in situ hybridization (FISH) analysis performed on the marrow at the time of the initial diagnosis of MDS-EB1 was negative for recurring MDS-related chromosome abnormalities, including deletion or loss of chromosomes 5, 7, 13, 17, and 20; gain of chromosome 8; and *KMT2A (MLL)* translocations. At MSKCC, karyotype analysis of the AML bone marrow aspirate specimen showed a normal male karyotype, and FISH analysis was negative for the presence of *KMT2A (MLL)*, *EVI1*, and *CBFB* translocations, and *TP53* deletion. A targeted next-generation sequencing (NSG) panel (MSK-IMPACT heme panel; MSKCC, New York, NY) was performed on the bone marrow specimen obtained at the time of the AML diagnoses, which showed the alterations listed in Table 1, including mutations in *ASXL1, KMT2D, RUNX1, SF3B1, TET2, PHF6, RAD21,* and *XBP1*. Evaluation of this bone marrow sample using an anchored multiplex polymerase chain reaction–based, targeted RNA sequencing assay for detection of fusions (Archer FusionPlex, ArcherDx, Boulder, CO) revealed the presence of a fusion between exon 1 of *P2RY8* and exon 1 of *CRLF2* (Fig 1C). This fusion was confirmed in 22% of cells using FISH break-apart probes for *CRLF2* and *P2RY8* (Xp22.33/Yp11.32; Cytocell, Tarrytown, NY; Fig 1D). The signal pattern was consistent with an interstitial deletion between the *P2RY8* and *CRLF2* genes within the pseudoautosomal region 1 (PAR1).

To ensure that the *P2RY8-CRLF2* rearrangement observed was present in the myeloid blasts rather than cells of other lineages, bone marrow mononuclear cells were separated by fluorescence-activated cell

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Is there utility to fusion discovery of known fusions in novel disease contexts?

Knowledge Generated

P2RY8–cytokine receptor–like factor 2 (*CRLF2*) fusions have been observed in B-acute lymphoblastic leukemia but we report, to our knowledge, the first detection of this fusion in acute myeloid leukemia arising from myelodysplastic syndrome. Cell sorting, molecular characterization, and immunostaining characterized the myeloid origin of this fusion and subsequent signaling effects.

Relevance

This patient was treated with the novel combination of decitabine and ipilimumab with an outstanding response. Immune cell infiltrates in the bone marrow with treatment suggests the response was related to the immune checkpoint inhibition. Additional study of *P2RY8-CRLF2* fusions and response to immunotherapy is warranted and could have implications for fusion discovery in novel disease contexts.

sorting to isolate the leukemic blasts, myelomonocytic cells, and normal lymphocytes. FISH using the *CRLF2* break-apart probe revealed the *CRLF2* rearrangement in a subset of the myeloid blasts (27.5%) and myelomonocytic cells (19.5%) but not in B cells (Fig 2).

FISH evaluation of the prior bone marrow biopsy with MDS-EB1 was also performed, which showed the presence of the *P2RY8-CRLF2* rearrangement in 10% of the cells (Appendix Fig A1C). Archer FusionPlex of the prior marrow with MDS-EB1 also identified the same fusion between exon 1 of

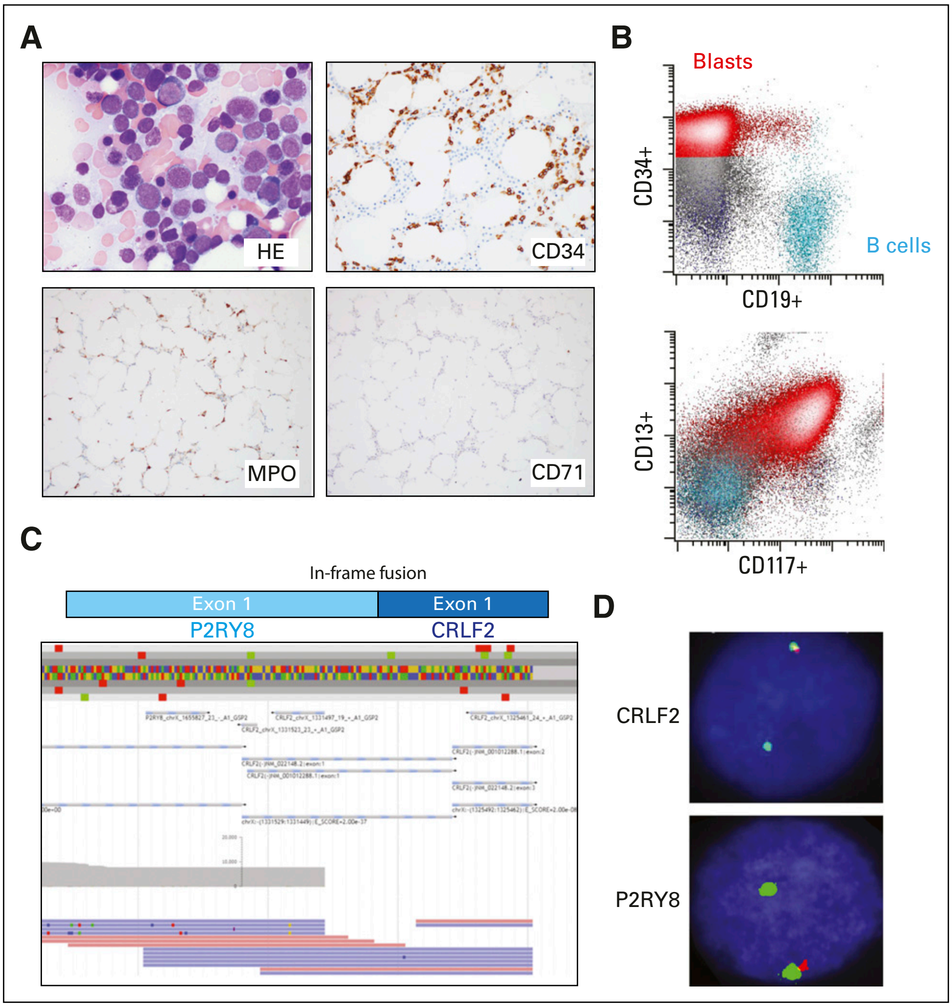

FIG 1. Acute myeloid leukemia (AML) with myelodysplasia-related changes with *P2RY8*–cytokine receptor–like factor 2 (*CRLF2*) fusion. (A) Photomicrographs of the bone marrow biopsy showing AML. (B) Flow cytometry of the bone marrow aspirate showing typical AML markers. (C) Anchored multiplex polymerase chain reaction (Archer FusionPlex) reads showing an in-frame fusion between *P2RY8* and *CRLF2*. (D) Break-apart fluorescence in situ hybridization probes for *CRLF2* (top) and *P2YR8* (bottom) consistent with interstitial deletion between the 2 genes resulting in fusion.

TABLE 1. Comparison of Molecular Findings in the Patient's Marrow Samples Obtained at the Time of MDS-EB1 and AML-MRC

	VAF %	
Mutational Profiling	**MDS-EB1 Marrow (May 2017; %)**	**AML-MRC Marrow (Sep 2018; %)**
ASXL1 c.1900_1922del (p.E635Rfs*15)	14.4	19.8
KMT2D c.10505_10506insTTTACCC (p.N3503Lfs*4)	5.5	7.6
RUNX1 c.497G>A (p.R166Q)	5.8	17.5
SF3B1 c.1774G>A (p.E592K)	25.6	18.9
TET2 c.4138C>T (p.H1380Y)	8.3	17.1
SETD1B c. 2502G>A (p.W834*)	7.7	<1.0
PHF6 c.673C>T (p.R225*)	0.0	20.2
RAD21 c.371_372insTGAGGGCGGA (p.L124Ffs*6)	0.0	5.7
XBP1 exon 1 deletion	Not present	Present (VAF not available)

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; MDS-EB1, myelodysplastic syndrome with excess blasts-1; VAF, variant allele frequencies.

P2RY8 and exon 1 of *CRLF2* (Appendix Fig A1B). OncoScan array (ThermoFisher, Waltham, MA) of the MDS-EB1 biopsy did not reveal any unbalanced genomic changes or copy-neutral loss of heterozygosity (data not shown), consistent with the normal FISH study observed by the outside institution a year prior. Targeted NGS sequencing (MSK-IMPACT heme panel) of the MDS-EB1 bone marrow specimen also showed overlapping mutations with the AML bone marrow sample (Table 1), but with acquisition of new mutations (*PHF6, RAD21, XBP1*) in the AML sample, suggesting a myeloid primed hematopoietic stem cell/progenitor origin and clonal evolution with expansion of the clone with the *P2RY8-CRLF2* rearrangement, likely due to acquisition of a loss-of-function mutation in *PHF6*, which led to down-regulation of genes involved in normal B-cell development.[4]

Total RNA sequencing of the AML bone marrow specimen further confirmed the presence of the *P2RY8-CRLF2* fusion along with overexpression of CRLF2 (Fig 3A). Principal component analysis comparing our patient with cohorts of AML or B-ALL from the TARGET study[5] showed that the patient's neoplasm clustered with other patients with AML, confirming the myeloid differentiation of the blasts (Fig 3B). Gene ontology analysis showed transcriptome-wide increases in cytokine-related signaling pathway genes potentially related to the *P2RY8-CRLF2* fusion, as well as genes involved in regulating hematopoietic progenitor cell differentiation and the spliceosome (Fig 3C).

Because it is known that STAT5 is activated by *P2RY8-CRLF2* fusion in B-ALL, we performed immunohistochemistry (IHC) using phosphorylation-specific antibodies that showed increased STAT5 phosphorylation and partial ERK phosphorylation but not STAT3 phosphorylation (Fig 4A). Gene expression and IHC were largely correlated; however, the results are interpreted in the context of the importance of signaling in leukemia overall, and these are not necessarily specific for *P2RY8-CRLF2* fusion.

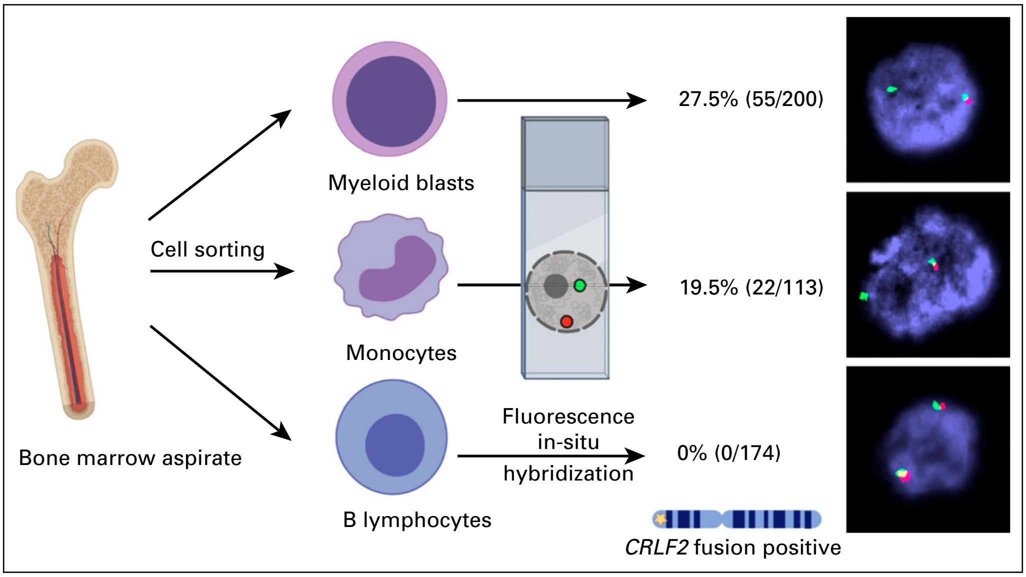

FIG 2. Confirmation of *P2RY8*–cytokine receptor–like factor 2 (*CRLF2*) fusion in myeloid lineage. The bone marrow aspirate sample was sorted by fluorescence-activated cell sorting into the myeloid blast population, myelomonocytic cells, and B lymphocytes. Break-apart fusion probes were applied to the sorted populations showing restriction of the *P2RY8-CRLF2* fusion to the myeloid lineage, including the blasts and myelomonocytes.

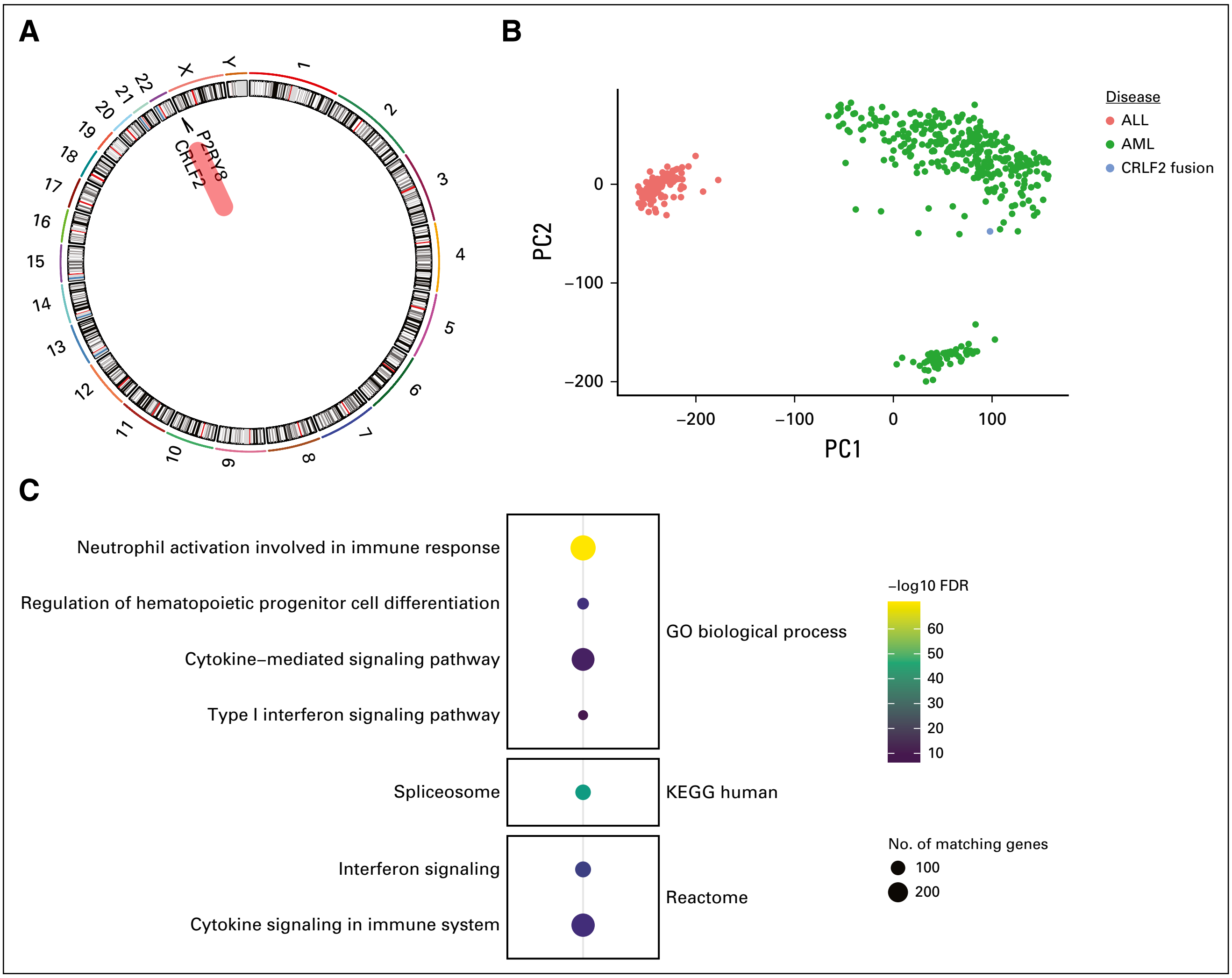

FIG 3. Transcriptomic analysis of *P2RY8*–cytokine receptor–like factor 2 (*CRLF2*) fusion AML. (A) Fusion analysis performed from polyA selected mRNA next-generation RNA sequencing. (B) Principal component analysis plot showing 2 strongest principal components (PC1 on *x*-axis and PC2 on *y*-axis) from publicly available data from AML (green dots) and B-ALL (red dots) patient samples compared with our patient with *P2RY8-CRLF2* fusion (blue dot). (C) Gene ontology analysis of the most highly expressed genes showing enrichment in hematopoietic differentiation, spliceosome, and immune signaling gene pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

TREATMENT AND FOLLOW-UP

Given the patient's age and transformation to AML, a clinical trial was recommended. He was consented and enrolled in a study regimen consisting of standard-dose decitabine plus investigational administration of the cytotoxic T-lymphocyte–associated protein-4 (CTLA-4) antagonist, ipilimumab (ClinicalTrials.gov identifier: NCT02890329). After 2 cycles of combination therapy, he achieved complete remission with incomplete count recovery and shortly thereafter a full complete remission (Table 2) with negative measurable residual disease (MRD) status by a flow cytometric assay with a detection limit of 1 in 2,500 cells. This was accompanied by a robust molecular response with undetectable levels of the previously detected *PHF6, RUNX1, TET2*, and *RAD21* mutations, and a marked reduction in the variant allele frequencies for the mutations *ASXL1* (0.4%) and *SF3B1* (1.4%), likely representing MRD below the detection limit of flow cytometry. The patient continues to be in morphologic remission and in cycle 11 of investigational therapy at the time of this report. IHC was performed on the checkpoint molecules including programmed death (PD)-1, PD-ligand 1 (PD-L1) and PD-L2 (Fig 4B). The PD-1 stain was positive in scattered T cells. Although the PD-L1 stain showed equivocal results, gene expression by RNA sequencing was at the 75th percentile compared with other profiled AML. Multiplexed immunofluorescence with a panel of antibodies against CD34, CD3, CD4, CD8, and granzyme B (GZMB), as previously described,[6] was applied to the patient's pretreatment bone marrow, which revealed the presence of a dense tumor immune infiltrate that was CD8+ GZMB+, suggesting his marrow was enriched with activated CD8 T cells critical for response to checkpoint blockade (Fig 4C).

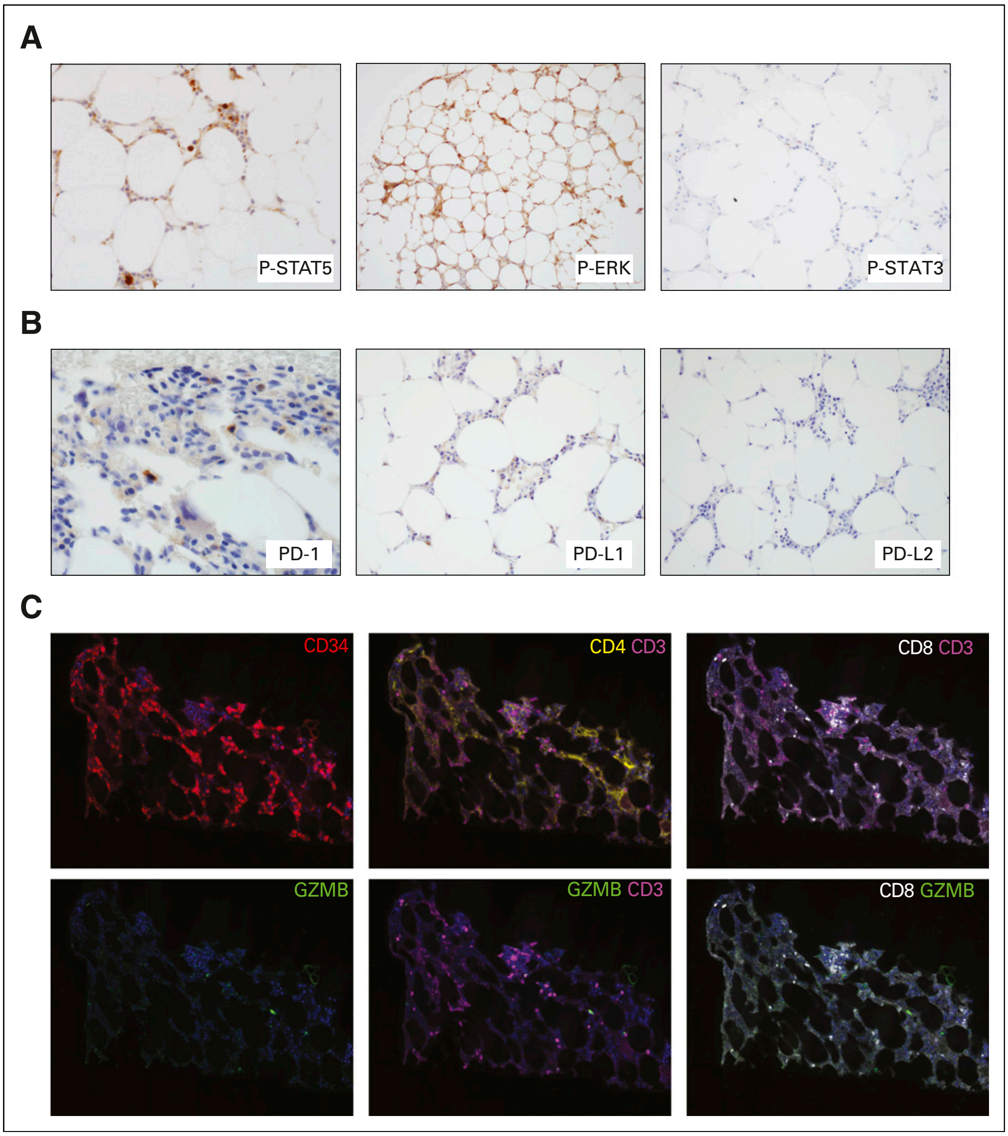

FIG 4. Immune signaling activation in *P2RY8*–cytokine receptor–like factor 2 (*CRLF2*) fusion AML. (A) Immunohistochemical photomicrographs of the bone marrow biopsy showing phospho-specific staining of STAT5 and ERK, but not STAT3. (B) Immunohistochemical photomicrographs of the bone marrow biopsy showing staining of programmed death (PD)-1 in scattered lymphoid cells. PD-ligand 1 (PD-L1) staining was equivocal, whereas PD-L2 was negative. (C) Using multiplex immunofluorescence microscopy, with digital image analysis, we found that the patient's bone marrow was highly enriched with CD8 plus granzyme B (GZMB) plus T cells. CD34+ staining highlights the myeloblast population at the time of AML transformation. We further identified a population of CD3+CD4+ T cells at baseline.

DISCUSSION

CRLF2 is the protein product of a synonymous gene located in the PAR1 on the short arm of the X chromosome and the Y chromosome. It forms a heterodimer with interleukin 7 receptor alpha and is involved in lymphoid signaling pathways. Activation of this receptor leads to activation of Janus family tyrosine kinases and STAT5. Deregulated expression of this gene has been described as the characteristic alteration in Philadelphia chromosome–like B-ALL in adolescent and adult patients.[7,8] Russell et al[9] showed that this subgroup of B-ALL has either a translocation juxtaposing the *CRLF2* to the *IGH* gene on chromosome 14 (t(X;14)(p22;q32) or t(Y;14)(p11;q32)) or a deletion of PAR1 region, resulting in overexpression of the *CRLF2* gene. Mullighan et al[10] reported that the interstitial deletion of PAR1 leads to juxtaposition of *CRLF2* to the first, noncoding exon of *P2RY8*. This event was seen in up to 7% of childhood B-ALLs and in up to half of patients with Down syndrome presenting with ALL.

Dysregulated expression of CRLF2 has been shown to occur as both early events (founder or truncal alteration),

TABLE 2. Complete Blood Count and Bone Marrow Parameters Over Time

Parameter (range)	MDS Diagnosis	AML-MRC Diagnosis	Post Cycle 2	Post Cycle 4
WBC K/μL (4.0-11)	2.3	0.8	6.8	4.5
Hgb g/dL (12.5-16.2)	13.6	8.0	9.6	12.8
PLT K/μL (150-400)	40	54	104	143
ANC K/μL (1.5-7.5)	1.4	0.2	0.24	1.4
BM blasts (%)	8	40	< 5	2

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; ANC, absolute neutrophil count, BM, bone marrow; Hgb, hemoglobin; MDS, myelodysplastic syndrome; PLT, platelets.

mostly seen as *IGH-CRLF2* fusion, and late events, often observed as *P2RY8-CRLF2* fusion. This has been observed in Down syndrome–related ALL as well as non–Down syndrome ALL, supporting the cooperation of *CRLF2* events with other genetic alterations in progression of disease.[11] However, irrespective of the clone size, presence of this rearrangement is associated with a higher relapse rate.[12] There have been suggestions that the increased relapse rate may be due to other concurrent alterations, such as *IKZF1* deletion.[13] However, other studies have shown the rearrangement as well as the CRLF2 overexpression to be independent predictors of worse outcome in B-ALL.[14,15]

Until now, only one patient with myeloid neoplasm with CRLF2 overexpression has been reported. However, this patient was associated with multiple copies of an isodicentric Y chromosome and not associated with *P2RY8-CRLF2* rearrangement.[3] To our knowledge, our patient represents the first *P2RY8-CRLF2* rearrangement in myeloid neoplasms, which in our patient was an AML arising from the underlying MDS. Although this rearrangement was previously thought to originate from B lymphocytes because it was only observed in B-ALL, this was not the case for our patient. The flow-sorted FISH analysis has shown that the *P2RY8-CRLF2* was not present in the B lymphocytes of this patient but only in the myeloid-lineage cells (myeloid blasts and myelomonocytic cells). In addition, based on the results from total RNA sequencing, our patient's neoplasm clustered with other patients with AML, providing additional evidence for the myeloid differentiation of the blasts. Furthermore, the alterations observed in this patient, including the *P2RY8-CRLF2* rearrangement, were present at the MDS stage, suggesting a stem cell origin with selective pressure leading to development of AML.

PHF6 mutations are rare in de novo AML but relatively common in AML-MRC and exceedingly rare in B-ALL.[16,17] *PHF6* and *DNMT3A* are the most common mutations in mixed phenotype acute leukemias (MPAL) with T-lineage differentiation.[18] PHF6 is a critical component in lineage determination of precursor cells, especially between T and B lineages, and it is believed that a functioning PHF6 is required for B-cell differentiation leading to selective abundance of this mutation in T-ALL and MPAL with T-lineage differentiation.[18,19] This notion is further supported by the observation that PHF6 suppression leads to impaired tumor progression in B-ALL.[4] It has also been shown that PAX5-deficient pro-B cells can undergo myeloid-lineage differentiation in the presence of transcription factors, such as GATA or CEBPA.[20] In our patient, the immunophenotype and RNA expression data unequivocally point toward myeloid differentiation. Although it is difficult to ascertain the functional consequences of the *P2RY8-CRLF2* rearrangement and the acquired *PHF6* mutation at the time of AML transformation, we hypothesize that the two may interact in a way that led to the development of AML instead of B-ALL.

Remarkably, this patient with AML-MRC and *PHF6*, *RUNX1*, *TET2*, and *RAD21* mutations had an MRD-negative complete response by flow cytometry after just 4 cycles of combination decitabine with ipilimumab. Although the complete results from clinical trials of these agents are needed to evaluate the efficacy and safety of this combination, this patient's exceptional response could suggest the unique molecular characteristics of his tumor may have been important, namely, the *P2RY8-CRLF2* fusion. Recent evidence in head and neck cancers with oncogenic chromosomal rearrangements suggests that gene fusions may be a source of immunogenic neoantigens and stimulate T-cell responses that could be unleashed after immune checkpoint blockade.[21] This patient's T cells were positive for PD-1, suggesting a relation to the response to CTLA-4 blockade. Additional research is needed to define whether this mechanism of action could be at play with other fusions or in the case of subclonal alterations, such as in this patient.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

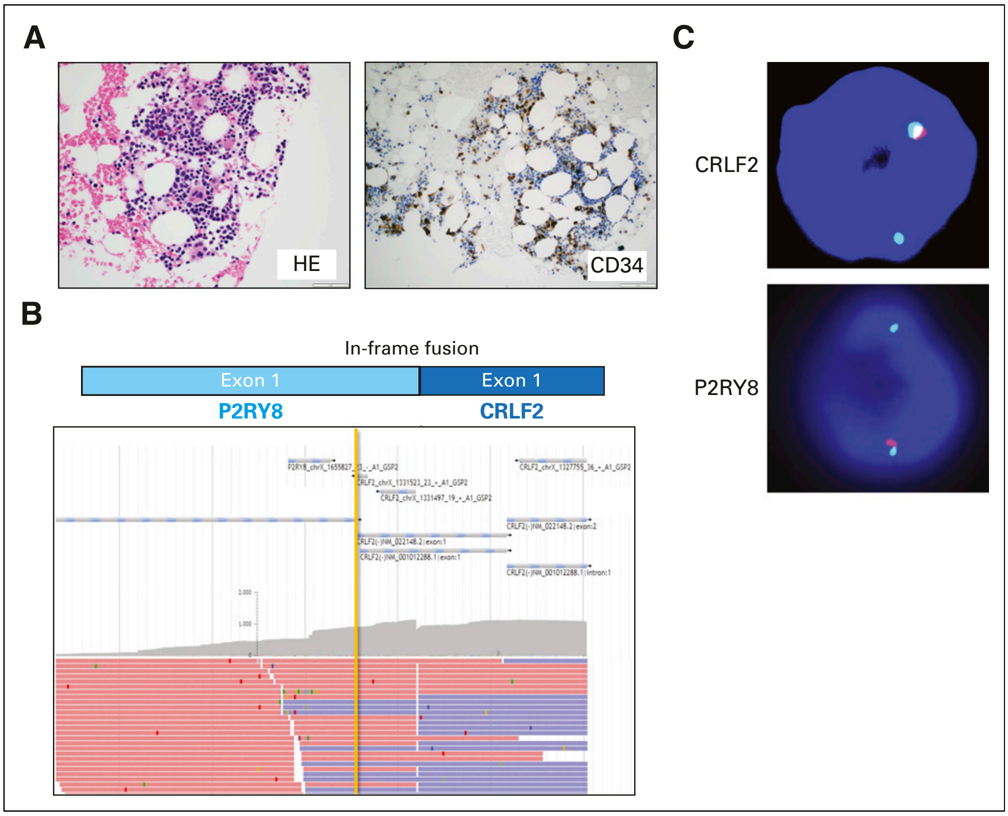

FIG A1. Presence of the *P2RY8-CRLF2* fusion in earlier bone marrow biopsy with myelodysplastic syndrome (MDS). (A) Photomicrographs of the bone marrow biopsy showing MDS with excess blast-1 (MDS-EB1). (B) Anchored multiplex polymerase chain reaction (Archer FusionPlex) reads showing an in-frame fusion between *P2RY8* and *CRLF2*. (C) Breakapart fluorescence in situ hybridization probes for *CRLF2* (top) and *P2YR8* (bottom) consistent with interstitial deletion between the 2 genes, resulting in fusion.