Chemotherapy Resistance in B-ALL with Cryptic NUP214-ABL1 Is Amenable to Kinase Inhibition and Immunotherapy

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Abstract

BCR-ABL1 kinase inhibitors have improved the prognosis of Philadelphia-chromosome-positive (Ph+)-acute lymphoblastic leukemia (ALL). Ph-like (or BCR-ABL1-like) ALL does not express BCR-ABL1 but commonly harbors other genomic alterations of signaling molecules that may be amenable to therapy. Here, we report a case with a NUP214-ABL1 fusion detected at relapse by multiplexed, targeted RNA sequencing. It had escaped conventional molecular work-up at diagnosis, including cytogenetic analysis and fluorescence in situ hybridization for ABL1 rearrangements. The patient had responded poorly to initial multi-agent chemotherapy and inotuzumab immunotherapy at relapse before the fusion was revealed. The addition of dasatinib targeting NUP214-ABL1 to inotuzumab resulted in complete molecular remission, but recurrence occurred rapidly with dasatinib alone. However, deep molecular remission was recaptured with a combination of blinatumomab and ponatinib, so he could proceed to allotransplantation. This case illustrates that next-generation sequencing approaches designed to discover cryptic gene fusions can benefit patients with Ph-like ALL.

Key words: Ph-like; BCR-ABL1 l-like; acute lymphoblastic leukemia; NUP214-ABL1; cryptic translocation.

Key Points
• NUP214-ABL1 rearrangements that are cryptic in conventional diagnostics can contribute to treatment resistance in Ph-like B-ALL and may be revealed by next-generation sequencing assays.
• Therapy with combined dasatinib and inotuzumab can overcome treatment resistance associated with NUP214-ABL1 translocation.
• Therapy with ponatinib and blinatumomab can revert MRD recurrence on dasatinib in NUP214-ABL1+ ALL.

Patient Story

A 34-year-old male migrant worker from Central America initially presented to another academic hospital with leukocytosis of 29.9 × 10^9/L with 65% blasts. The bone marrow exhibited ~80% blasts with the following immunophenotype determined by flow cytometry: CD19+, CD10+, CD22+, cytoplasmic CD79a+, TdT+, CD34+ CD20-. Cytogenetic studies demonstrated 2 unrelated abnormal clones in 14 of 19 metaphases analyzed, 46,XY,+1,der(1;22)(q10;10) in 9 cells, and 46,XY,add(7)(q31),i(8)(q10),der(16)(t;16)(q12;p13.3) in 5 cells. In addition, fluorescence in situ hybridization (FISH) for BCR-ABL1 and KMT2A rearrangements was negative, as was a BCR-ABL1 PCR assay. Accordingly, he was treated for Ph-negative B-ALL utilizing a pediatric multi-agent chemotherapy regimen (CALGB 10403).1 After induction course 1 (intrathecal cytarabine, prednisone, vincristine, daunorubicine, pegylated asparaginase, intrathecal methotrexate), he went into complete remission (CR), albeit flow cytometry revealed measurable residual disease (MRD) (2% of the nucleated bone marrow cells). The CALGB 10403 regimen as published advises the use of extended remission induction (further prednisone, daunorubicin, vincristine, pegylated asparaginase) for patients after-induction with >1% lymphoblasts,1,2 but he immediately received consolidation with course 2 (cyclophosphamide, cytarabine, 6-mercaptopurine, vincristine, pegylated asparaginase, intrathecal methotrexate), was discharged after count recovery and was lost to follow up.
Fourteen weeks later, he presented to the second hospital’s emergency department with malaise, nosebleeds, and bruising. He had a platelet count of $<5 \times 10^9/L$, hemoglobin of 6.1 g/dl, and leukocytosis of $212 \times 10^9/L$ with 89% B lymphoid blasts, now weakly CD20+. Cytogenetic analysis from the peripheral blood was unsuccessful, and FISH analysis was negative for a BCR-ABL1 rearrangement. Sequencing of amplicons from genomic DNA for 68 candidate genes (Illumina TrueSeq Amplicon Assay) revealed mutations in KRAS (p.Gly12Asp, variant allele frequency [VAF] 47%) and in EZH2 (p.Ser695Leu, VAF 72%). He was treated with inotuzumab ozogamicin (0.6 mg/m$^2$, day 2 and 0.3 mg/m$^2$, day 8) in combination with Mini-hyper-CVD (hyperfractionated cyclophosphamide, vincristine, dexamethasone) and rituximab as described. 3 This resulted in the eradication of blasts from the peripheral blood and transient pancytopenia, and he was discharged on day 28 to establish care in Boston.

He presented to our ambulatory clinic feeling well. His peripheral blood exhibited no blasts and was otherwise normal except for mild anemia (hemoglobin 9.9 g/dl, platelet count 204 $\times 10^9/L$, total white blood cell count 4.17 $\times 10^9/L$ with normal differential). Flow cytometry analysis and cytology of the CSF showed no evidence of B-ALL. However, bone marrow biopsy core sections revealed decreased normal hematopoietic elements, a cellularity of 70% and 70% lymphoid blasts (Fig. 1A). The aspirate smear showed 48% blasts, and flow cytometric analysis of the bone marrow aspirate confirmed 41% CD20+ B lymphoid blasts. These findings established persistent, relapsed B-ALL.

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We confirmed the absence of a BCR-ABL1 fusion by cytogenetic analysis, PCR, and FISH. A 47,XY,+7, add(7)(q32q34), i(8)(q10), ps dic(16;1)(p13.2;p11) karyotype was observed in 18 of 20 metaphases analyzed (Fig. 1B).

Sequencing of bone marrow confirmed mutations in KRAS (p.Gly12Asp, VAF 34.4%) and in EZH2 (p.Ser695Leu, VAF 70.9%), both pathogenic. Intriguingly, bioinformatic analysis of sequencing reads uncovered focal loss within IKZF1 corresponding to the dominant-negative isoform IK6 that lacks its DNA-binding domain (exons 4-7) but retains the dimerization domain and functions as a dominant negative (Fig. 1C,D). IKZF1 mutations are common in Ph+ ALL, and in Ph-like ALL, an entity that resembles Ph+ALL in regard to downstream pathway activation, gene expression as well as poor prognosis.6,7 Ph-like ALL commonly harbors genomic alterations8 which can be targeted to improve therapeutic outcomes.7,9

To allow for comprehensive searches for pathogenic gene fusions in clinical samples, we used a clinically validated, targeted RNA-based assay that utilizes the next-generation sequencing of amplicons generated with anchored multiplex...
PCRs (heme fusion assay). This assay is designed to capture hybrid transcripts involving 82 target genes that have been previously implicated in gene aberrant fusions. Using this approach, we confirmed the significant expression of IK6 (data not shown). More importantly, we detected NUP214-ABL1 chimeric transcripts, in which exon 32 of NUP214 is joined to exon 3 of ABL1 (Fig. 1E). NUP214-ABL1 may result from a small tandem duplication of chromosome 9q (q34q34) that is cytogenetically cryptic by G-banding and by FISH analysis using break-apart FISH probes for ABL1 (Fig. 1F). Amplification of ABL1 may be apparent in FISH as a result of episomal replication of NUP214-ABL1 but in this case FISH for ABL1 did not reveal amplification (Fig. 1G). In NUP214-ABL1, the predicted hybrid protein joins the N-terminal part of NUP214, featuring an oligomerization motif, to the C-terminal portion of ABL1 generating a constitutively active tyrosine kinase. NUP214-ABL1 was first identified in up to 6% of T-cell ALL cases, and is now a well-established driver in this context. While activation of the ABL1 kinase in such cases resembles the better-known BCR-ABL1, these cases of T-ALL are not designated Ph-like, as this entity was discovered and defined in B-ALL. Moreover, BCR-ABL1 occurs exceedingly rarely in T-ALL. More recently, however, at least 12 B-ALL cases with NUP214-ABL1 have been reported, ranging from childhood to young adulthood (age/sex: 12M, 13F, 14M, 15F, 16M, 17M, 18M, 22M, 24F, 26F, 31F). In most of these cases, the breakpoints in the ABL1 gene have been localized to exon 2 similar to the vast majority of CML and Ph+ALL cases. However, in 3 of these 12 cases, the breakpoint has been localized to ABL1 exon 3 just like we demonstrated in our case (Fig. 1E). Notably, ABL1 exon 3 fusions have also been documented in rare cases of Ph-B-ALL and CML and appear to function largely like the more common exon 2 fusions in the context of BCR-ABL1. Given that 4 among 13 (including this one) reported cases of NUP214-ABL1-B-ALL to involve ABL1 exon 3, such ABL1 exon 3 fusions may be more common with NUP214 and generate active disease drivers. Most of the 12 previously reported cases of NUP214-ABL1-B-ALL were associated with mutant IKZF1, including the IK6 isoform, similar to the case reported here. Importantly, in all 10 cases with reported clinical follow-up, MRD was not abolished after induction chemotherapy without targeting the fusion oncogene, and 5 cases followed to achieve remission. Importantly, downstream signaling by the hybrid kinase and responsiveness to dasatinib was demonstrated in experimental models. Moreover, early data suggest the clinical utility of ABL1 kinase inhibitors in NUP214-ABL1-B-ALL. Dasatinib likely contributed to a transient (~3 months) remission in a chemotherapy-refractory case relapsed after transplant. In another case, the addition of dasatinib to a second cycle of intensive chemotherapy failed to eradicate MRD pretransplant, but its continuation posttransplant may have contributed to eliminating MRD after 20 months. Finally, in a recent series, the addition of dasatinib or imatinib to chemotherapy likely contributed to the reduction or eradication of MRD before transplantation in 4 out of 5 cases. Three of these cases remained in remission 11, 43, and 45 months after transplant at the time of reporting. Notably for NUP214-ABL1-B-ALL prolonged remissions have only been reported in cases that were treated with both ABL1 kinase inhibitors and stem cell transplant. These observations suggest that integrating targeted agents into treatment strategies may be as critical for improving clinical results in Ph-like ALL as is already established in Ph+ALL.

**Patient Update**

For our patient, in light of the persistent extensive bone marrow involvement after one cycle of chemo-immunotherapy (Fig. 1A) and the discovery of the NUP214-ABL1 fusion (Fig. 1E), we elected to continue treatment with inotuzumab (0.8 mg/m² on day 1, 0.5 mg/m² on days 8 and 15) but replaced systemic cytotoxic chemotherapy with dasatinib (140 mg by mouth per day starting on day 9) (Fig. 2A). With this, the patient did not require transfusions or become neutropenic. Remarkably, a bone marrow biopsy (bmbx) on day 29 showed normal hematopoiesis (Fig. 2A, bmbx2), no evidence of leukemia by morphology and flow cytometry (sensitivity 1 × 10⁻⁶), normal cytogenetics, and absence of detectable mutations on NGS sequencing panels. Moreover, NGS-based MRD-tracking using 3 clonal immunoglobulin heavy chain locus sequences, showed undetectable leukemia (sensitivity 1 × 10⁻⁴, clonoSEQ, Adaptive Biotechnologies) (Fig. 2B). At this point, inotuzumab was discontinued because of the increasing VOD risk with higher cumulative doses, and the patient was closely monitored on dasatinib while expediting transplantation. However, on day 78 of dasatinib, we detected MRD recurrence (0.8% by flow cytometry; 0.53% by NGS (clonoSEQ)) (Fig. 2A and B, bmbx3). Together, these data revealed that the combination of inotuzumab and dasatinib has marked activity in relapsed NUP214-ABL1-B-ALL. They likely work additively or synergistically as the combination rapidly produced a complete molecular remission. In contrast, inotuzumab alone had only modest activity (see above, patient story) and dasatinib alone failed in maintaining this response. MRD recurrence in remission of ALL is ominous and predicts overt relapse after a median of 3 months in 80% of the cases. To address this, we started CD19-directed therapy with blinatumomab, highly effective in eradicating MRD, and switched dasatinib to ponatinib (45 mg by mouth per day) given prior data of its activity in treating dasatinib-resistant Ph+ALL. Recent data suggest that the combination of these agents shows higher response rates and duration in relapsed Ph+ALL compared with either single agent. Indeed, 4 weeks after this therapeutic change, MRD was absent by flow cytometry and NGS (Fig. 2A, B, bmbx4). We cannot formally conclude that both agents were required for this response. Nevertheless, this data demonstrates for the first time that the combination of blinatumomab with ponatinib has the potential to effectively treat dasatinib-resistance manifesting as MRD recurrence in NUP214-ABL1-B-ALL. Specific data on the impact of stem cell transplantation on NUP214-ABL1-B-ALL or Ph-like ALL after relapse is lacking, but the published experience with Ph+ALL is a reasonable surrogate for such data. The 5-year overall survival after allograft transplantation for relapsed Ph+ALL is only 3%-10%, but stem cell transplantation is the sole treatment option with a track record of long-term survival in this context. There is hope that the integration of novel agents into therapy will eventually improve these dismal outcomes. The combination of blinatumomab with ponatinib appears promising and achieved a high complete response rate in relapsed Ph+ALL and subsequent allotransplantation was associated with a significant reduction of the relapse rate after such
remissions (12.5% vs 82.3%). Given these data and the short duration of the responses to this patient’s prior treatments, allotransplant appeared to be the only curative option at this point.

He thus proceeded to a myeloablative (cyclophosphamide/total body irradiation) peripheral blood stem cell transplant from a fully HLA-matched unrelated donor in deep remission. Unfortunately, he succumbed to complications of severe intestinal graft versus host disease 4.5 months after transplant with the ALL in continued remission. Our case highlights that NUP214-ABL1 + B-ALL can be revealed with next-generation sequencing-based assays while it may escape conventional cytogenetic analysis and FISH. Early diagnosis will allow for integrating potent novel agents into therapy regimens and may improve outcomes.

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Conflict of Interest
Gabriela S. Hobbs: Takeda (C/A); Andrew M. Brunner: Acceleron, Agios, Celgene/BMS, Novartis, Takeda (C/A), Celgene/BMS, AstraZeneca, GlaxoSmithKline, Janssen, Novartis, Takeda (RF); Philip Amrein: ASTEX, Millennium/Takeda, Amgen, AstraZeneca (RF--inst), AstraZeneca (SAB); Amir T. Fathi: Daiichi Sankyo, Novartis, Celgene/BMS, Kite, Trovagene, Forty Seven, Newlink Genetics, Pfizer, Abbvie, Genentech, Astellas, Blueprint, Kura, Trillium, Takeda, Amgen, Agios, Seattle Genetics (C/A), Celgene/BMS, Agios, Takeda, Abbvie (RF). The other authors indicated no financial relationships.

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Author Contributions
Conception/Design: V.N. and H.H. Provision of study material or patients: V.N. and H.H. Collection and/or assembly of data: V.N. and H.H. Data analysis and interpretation: All authors. Manuscript writing: V.N. and H.H. Final approval of the manuscript: All authors.

Data Availability
The data underlying this article will be shared on reasonable request to the corresponding author.

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Figure 2. Treatments and response. (A) Clinical time course after diagnosis of relapsed ALL (months/days shown below axis). Bmbx: bone marrow biopsy. Mini-hyper-CVD: hyperfractionated cyclophosphamide, vincristine, dexamethasone. See results for details. (B) Next generation sequencing Tracing measurable residual disease (MRD) by next generation sequencing (ClonoSEQ, Adaptive Biotechnologies). At diagnosis (bmbx 1), 3 unique immunoglobulin heavy chain sequences were identified at high frequencies (>50% of nucleated cells). After treatment, MRD levels became undetectable (bmbx 2, detection limit: 1 cell/ million), eventually recurred (bmbx 3), but were abolished again after change in treatment (bmbx 4). See A, above and results for details.
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