Whole Genome Sequencing Uncovers Novel *BCR::ABL1* Breakpoints and Variants in Leukemia: Implications for Personalized Medicine

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INTRODUCTION

- The *BCR::ABL1* fusion gene is a hallmark of chronic myeloid leukemia (CML) and is present in a subset of acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and mixed phenotype acute leukemia (MPAL) cases.
- Traditional methods for identifying this important biomarker, such as FISH and RT-PCR, are limited in the ability to detect novel or complex variants.
- Whole genome sequencing (WGS) enables comprehensive detection of *BCR::ABL1* fusions and other genetic changes.
- Isoform identification (p190 vs. p210) informs leukemia classification and guides targeted treatment decisions; for example, p210 responds to imatinib, and the presence of ABL1 exon 2 is required for asciminib efficacy.

We sought to confirm *BCR::ABL1* chimeric transcripts identified by WGS-DNA using RNA sequencing (RNAseq), with the aim of improving diagnostic accuracy and informing isoform-specific therapeutic decisions in leukemia.

METHODS

We retrospectively analyzed 215 hematological clinical samples sent for testing at Tempus that received both WGS via Tempus xH and RNA-seq via Tempus xR. This unselected cohort of patients had a historical diagnosis of AML (n=146), MDS (n=41), CML (n=25), and other blood cancers (n=3). RNA was marked as true if direct or indirect count >=5 reads.

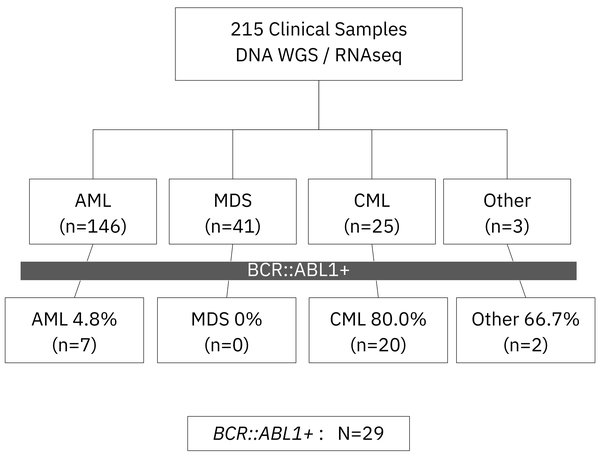


Figure 1. Diagram showing cohort diagnosis.

SUMMARY

- Our findings demonstrate that WGS can identify known BCR::ABL1 fusion events.
- WGS can uncover novel breakpoints and variants of clinical significance, particularly when used in conjunction with RNA-seq.
- These data suggest that WGS is a powerful tool for the comprehensive genomic profiling of leukemia.

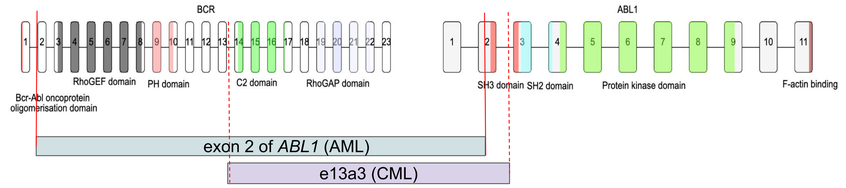
RESULTS

Figure 2. Cohort demonstrates disease specific DNA breakpoints in *BCR::ABL1*

A.

Cancer type	BCR category: p210 (Major)	p190 (Minor)
AML	1	5
CML	18	0
Heme Other	2	0

B.



C.

Figure 2. (A) Observed *BCR* category breakpoints by disease. All but 2 events have *ABL1* breakpoint in intron 1 (B) Gene representations with key domains indicated in *BCR* and *ABL1*. (C) The 2 cases that have atypical breakpoints in *ABL1*, one in exon 2 and another in intron 2.

Figure 3. WGS identifies 90% *BCR::ABL1* fusions found in deep targeted sequencing or RNA

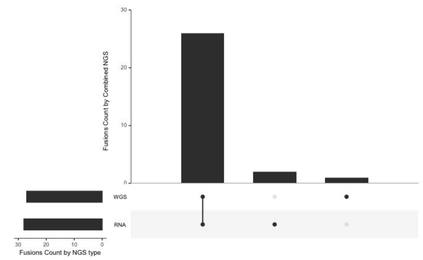


Figure 3. Confirmation of *BCR::ABL1* Structural Variants (SVs) by RNA Sequencing. WGS-identified SVs were confirmed by RNAseq in 26 of 29 cases (90%). Two SVs missed by WGS were detected only at very low RNA read counts, suggesting likely clonal events. In one specimen, WGS identified a *BCR::ABL1* fusion with the *ABL1* breakpoint located upstream of the gene body; although the fusion was detected in RNA, it was classified as “not matched” due to the conservative definition of WGS breakpoint assignment to the nearest intron.

Figure 4. WGS observed exon 2 and intron 2 breakpoint show RNA Exon 3 support.

A. *ABL1* pileup showing softclips for e13a3

B. *ABL1* pileup showing softclips for WGS breakpoint within exon 2

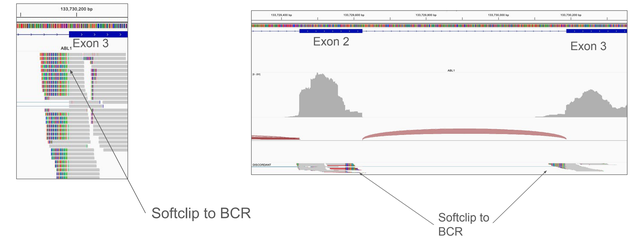


Figure 4. RNA supports WGS breakpoint and exon 3 usage (A) Softclip (reads support) to BCR exon 13/14 aligned to exon 3 in *ABL1* and (B) Exon 2 WGS breakpoint results in transcripts with loss of most exon 2 (shown by softclip reads) and transcripts that contain exon 3. Both examples result in loss of SH3 domain and suggest targeted therapeutic decision making.

Figure 5. ASXL1 is the most commonly mutated alteration within the matched WGS/RNA BCR::ABL1 cohort

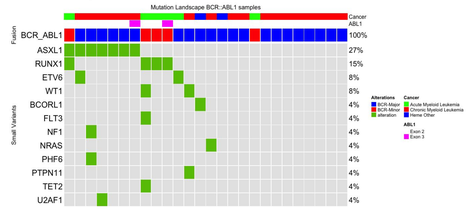


Figure 5. Of the 26 specimens positive for WGS and had RNA confirmation, 50% of them did not have any pathogenic small variants identified by WGS. *ASXL1* was the highest mutated genes in the cohort (27%) with *RUNX1* being mutated 19% of the time.

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