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Personalized measurable residual disease assays for acute myeloid leukemia guided by whole-genome sequencing

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Running head: Personalized MRD assays for AML guided by WGS

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Data availability

Raw data are available upon request at the discretion of the corresponding authors.

Author contributions

LWD and CSH were responsible for study conception and design. HE, MO, and JPR acquired and managed patient samples and clinical data. NR, RWA, JL, EvC, AC, and LWD developed assays and analysis pipelines. NR, RWA, CLD, LB, and LWD performed experiments and data acquisition. NR, RWA, JL, and LWD performed data analysis. RCL, JPR, LWD, and CSH provided study supervision. All authors wrote, reviewed, and/or revised the manuscript.

Competing interests

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Acute myeloid leukemia (AML) is an aggressive malignancy of the hematopoietic precursor cells. Oncogenic transformation occurring through the acquisition of structural aberrations is noted in ~35% of AML, and can result in the formation of fusion proteins that confer proliferation and survival advantages[1]. When compared to traditional cytogenetic testing for the identification of structural variants (SVs) or copy number variants (CNVs) at diagnosis in myeloid malignancies, newer techniques, like whole-genome sequencing (WGS), have been shown to increase diagnostic yield and refine risk stratification[2, 3]. A recent study revealed that half of AML patients with a normal metaphase karyotype had an SV/CNV of clinical significance identified by WGS[3]. Additionally, WGS can identify SV genomic breakpoints at a single basepair granularity, which is significantly finer compared to other standard methods (e.g. ~5Mb for karyotype, ~10-50Kb for microarray, ~100Kb for FISH) and the only genome-wide technique capable of this resolution.

Measurable residual disease (MRD) in complete remission (CR, <5% myeloblasts) is prognostic of outcomes in AML[4] and validated molecular methods for MRD monitoring are preferable to multiparametric flow cytometry (MFC), but are not available for all patients[5]. The limit of detection (LOD) of next-generation sequencing (NGS) assays for single nucleotide variants (SNVs) is restricted by technical error rates and not all patients have a molecular variant detectable by standard NGS assays. While SVs are common genetic features of AML, MRD approaches for detecting this class of variants have primarily relied on RNA. However, RNA has suboptimal stability, not all SVs are expressed as transcripts, and the impact of anti-leukemic therapy on transcription may make leukemic disease burden quantification inaccurate[6]. These shortcomings could largely be overcome by using DNA-based assays which provide accurate per-cell

quantification of MRD, although breakpoints are widely distributed in intronic regions[7]. In this study, we describe a sensitive DNA-based patient-personalized approach to MRD detection in AML by droplet digital PCR (ddPCR) using breakpoint information from WGS.

Previously, 62 adult patients with AML enrolled on the randomized Phase 3 SWOG-S0106 trial (NCT00085709) were assessed by our group for MRD in first CR by MFC and duplex sequencing (DS)[8]. While MRD detection by DS was highly predictive of adverse outcomes and strongly outperformed MFC, it did not always predict relapse and 8% of patients did not have an appropriate variant available for monitoring. Therefore, we sought to investigate if WGS-informed MRD tracking of SVs could overcome this limitation by selecting 5 patients from this cohort who experienced relapse and had known cytogenetic abnormalities (**Figure 1A, Table 1**). This proof-of-concept study consisted of 2 patients without and 3 patients with DS-trackable small variants. Among selected patients with trackable small variants, there were 2 false negatives and 1 true positive for MRD by DS, and all patients were MRD-negative by MFC. The Institutional Review Board of the Fred Hutchinson Cancer Center gave ethical approval for this work, and patients provided written and informed consent and were treated according to the Declaration of Helsinki.

For the 5 AML patients, genomic DNA and/or RNA was extracted from baseline and CR cryopreserved BM mononuclear cells (AllPrep, Qiagen). WGS libraries were constructed from baseline BM DNA using the TruSeq DNA PCR-free library preparation kit and subjected to 150-basepair paired-end sequencing at an average depth of 65X on the NovaSeq 6000 platform, per manufacturer's recommendations (Illumina). SVs (≥100Kb) and CNVs (≥5Mb) were identified

using the Chromoseq pipeline[2], which flags and retains recurrent and risk-defining SVs/CNVs while more stringent filtering criteria are applied to novel variants.

WGS successfully identified the genomic DNA breakpoints of all anticipated SVs in the 5 baseline AML samples (**Table 1**). In one patient (J9-11568), WGS was able to resolve an unclear cytogenetic finding on the q arm of chromosome (chr) 7 as a derivative chr resulting from a translocation between chr 7 and 11, der(7)t(7;11)(q31.1;q22.1), resulting in a 50Mb loss on 7q31.1-q36.3 and 35Mb gain on 11q22.1-q25. In a second patient (J9-11569), WGS identified a 2Mb deletion on 11q23.3. In both cases, the patient had core-binding factor AML harboring inv(16), and the identification of secondary cytogenetic abnormalities within this disease class has been linked to distinct prognostic impacts depending on the abnormalities identified[9].

One to two SVs were selected per patient and the breakpoints confirmed by Sanger sequencing **(Table 1, Supplementary Table 1).** Custom ddPCR assays targeting each SV were designed using primers flanking the DNA breakpoint and a FAM-tagged Taqman probe within the amplicon and multiplexed with a HEX-tagged *EIF2C1* reference gene assay (dHsaCP1000002, Bio-Rad) for calculation of wild-type genome copies. DNA was combined with the ddPCR assays, ddPCR supermix for probes (1863025, Bio-Rad), and HindIII, and droplet generation (Automated droplet Generator, Bio-Rad) followed by PCR amplification, and droplet reading (QX200, Bio-Rad) were performed per manufacturer's instructions. Data was analyzed using QuantaSoft Analysis Pro (ver. 1.0.596, Bio-Rad).

Initially, we tested this workflow using the genomic *CBFB::MYH11* breakpoints previously characterized in the ME-1 cell line[10]. Serial dilution of ME-1 cell line DNA into healthy donor DNA (range 5%-0.001%) established the limit of detection (LOD) as 0.001% variant allele fraction (VAF), with high sensitivity, no background noise, and high correlation to the expected VAF (r^2=0.99, P<0.0001) (**Supplementary Figure 1**). Individual patient assays were validated by serial dilution experiments (range 1%-0.0001%), establishing LODs ranging from 0.01%-0.001% VAF **(Figure 1B)**.

Patient-personalized assays were subsequently tested on post-treatment remission BM samples and identified MRD positivity in all 5 patients, with VAFs ranging from 0.0016%-0.016% (**Table 1**). This contrasted with DS, which predicted relapse in a single patient, and MFC which was negative for MRD in all patients. Patient J9-11578, who was MRD-positive by both DNA-based molecular methods had similar residual disease levels by each technique, with a VAF of 0.016% for the *DEK::NUP214* translocation by WGS-informed ddPCR and 0.011% for *FLT3*-ITD by DS. The remaining two patients with DS-trackable variants had residual SV VAFs of 0.0016% and 0.0022% for *CBFB::MYH11* and *RUNX1::RUNX1T1*, respectively. Two SV targets were tested for patient J9-11568, which revealed nearly identical residual SV VAFs for each event (0.0143% for *CBFB::MYH11* and 0.01385% for der(7)t(7;11)(q31.1;q22.1)).

Four of the 6 SVs tracked using DNA-based ddPCR assays have commercially available RNA-based qPCR MRD assays recommended by the ELN AML MRD guidelines[5] (*CBFB*::*MYH11* n=3, *RUNX1*::*RUNX1T1* n=1). Of the 4 patients, 2 patients had sufficient RNA for MRD assessment and tested positive by the gold standard qPCR MRD assays (*ipsogen*, Qiagen) (**Table 1, Supplementary**

Table 2), albeit with *ABL1* control gene expression less than the recommended level of 10,000 copies[5]. The ability to extract high-quality DNA but not always high-quality RNA for MRD assessment highlights one strength of DNA-based assays. Additionally, the remaining 2 SVs either did not have a commercially available RNA-based assay or do not produce an RNA transcript.

This study demonstrates a sensitive, patient-personalized strategy for AML MRD detection of SVs that uses breakpoint information from WGS and DNA as the analyte. In contrast to MFC, this approach successfully detected MRD in all remission samples in this cohort. For patients with an SV targetable by gold standard RNA-based techniques, WGS-informed ddPCR was concordant with qPCR or provided MRD evaluation when RNA quality was insufficient. Additionally, we were able to develop DNA-based molecular assays for patients without an RNA-based fusion or small variant targetable by standard approaches.

When comparing RNA- and DNA-based methods for AML MRD detection for common fusions, Lukes *et al* showed that DNA-based assays were more sensitive than RNA-based assays, and low transcript levels at diagnosis significantly affected MRD detection using RNA at later time points[11]. In another study performed on a cohort of pediatric AML patients, genomic MRD positivity at day 28 was the only significant predictor for overall and event-free survival by multivariate analysis[12]. Both studies used targeted NGS with custom probes capturing the fusions of interest. However, WGS can provide additional information regarding the diagnostic karyotype, such as CNVs in addition to genomic breakpoints. Moreover, copy number changes such as deletions of important tumor suppressor genes and passenger variants can also be potential MRD targets, which will

require future investigation. MRD detection by NGS is gaining wider acceptance, especially for *FLT3*-ITD and *NPM1* which are highly prognostic for relapse[13], and it would be reasonable to spike-in primers/probes targeting SVs into multigene NGS panels.

While WGS-informed MRD monitoring offers several benefits, some factors hindering its use in clinical laboratories would be the cost and expertise required to implement bioinformatics workflows, interpretation of results, and the regulatory burden of validating patient-specific assays. Furthermore, given the sensitivity of WGS[14, 15], tumor cell enrichment in AMLs with low blast percentages and/or deep WGS may be required. Additionally, while current evidence suggests there are few if any mutationally silent AML by WGS[3], the clinical utility of non-traditional MRD targets has yet to be established. Despite needing more extensive validation in larger patient cohorts to establish clinical utility and predictive MRD levels, the approach described here provides the opportunity to expand applicability of MRD testing to all AML patients.

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Table 1: Comparison of MRD assessment by WGS-informed ddPCR versus other techniques.

Patient	Baseline metaphase karyotype	Baseline WGS karyotype[1]	MRD result by MFC	DS MRD target	MRD result by DS, VAF	qRT-PCR MRD target	MRD result by qRT-PCR, NCN	WGS-informed MRD targets	MRD results by WGS-informed ddPCR[2], VAF
J9-11578	46,XX,t(6;9)(p23;q34)[20]	seq[GRCh38] t(6;9)(p22.3;q34.13) NC_000006.12:g.pter_18226504delins[NC_000009.12:g.131153507_qter] NC_000009.12:g.pter_131153503delins[NC_000006.12:g.18226235_qter]	Negative	*FLT3*-ITD	Positive, 0.011%	No target	Not evaluable	t(6;9)(p23;q34) *DEK::NUP214*	Positive, 0.0159%
J9-11574	46,XX,inv(16)(p13.1q22)[17]/47,XX,inv(16)(p13.1q22),+22[2]/46,XX[2]	seq[GRCh38] inv(16)(p13.1q22.1) NC_000016.10:g.[15721335_67085250inv;insT]	Negative	*NRAS* (G12S)	Negative	*CBFB::MYH11*	Not evaluable	inv(16)(p13.1q22) *CBFB::MYH11*	Positive, 0.0016%
J9-11576	46,XX,t(8;21)(q22;q22)[20]	seq[GRCh38] t(8;21)(q22.12;q21.3) NC_000008.11:g.pter_92066838delins[NC_000021.9:g.34844063_qter] NC_000021.9:g.pter_34844184delins[NC_000008.11:g.92066740_qter]	Negative	*KIT* (D816V)	Negative	*RUNX1::RUNX1T1*	Not evaluable	t(8;21)(q22;q22) *RUNX1::RUNX1T1*	Positive, 0.0022%
J9-11568	46,XY,del(7)(q3?4),inv(16)(p13.1q22)[22]	seq[GRCh38] der(7)t(7;11)(q31.1;q22.1), inv(16)(p13.1q22.1) NC_000016.10:g.[67088239_15721457inv;15721458del] NC_000007.14:g.108403332_qterdelins[GGCCCAGCAGT;NC_000011.10:g.99267776_qter]	Negative	No target	Not evaluable	*CBFB::MYH11*	Positive, 0.0019	inv(16)(p13.1q22) *CBFB::MYH11*; der(7)t(7;11)(q31.1;q22.1)	Positive, inv(16),0.0143% der(7),0.0139%
J9-11569	46,XX,inv(16)(p13.1q22)[21]	seq[GRCh38] del(11)(q23.3q23.3),inv(16)(p13.1q22.1) NC_000011.10:g.116763597_118773322del NC_000016.10:g.[15721403_67088894inv]	Negative	No target	Not evaluable	*CBFB::MYH11*	Positive, 0.0052	inv(16)(p13.1q22) *CBFB::MYH11*	Positive, 0.0040%

[1]Nomenclature following ISCN 2020 guidelines, genomic coordinates correspond to human genome build hg38

[2]Remission samples were tested by ddPCR using a total input of 900-1000ng of DNA distributed in 3-5 wells. Negative, positive, and no-template controls were included in each run. Samples were considered positive if fluorescence was detected above the LOD for the corresponding assay.

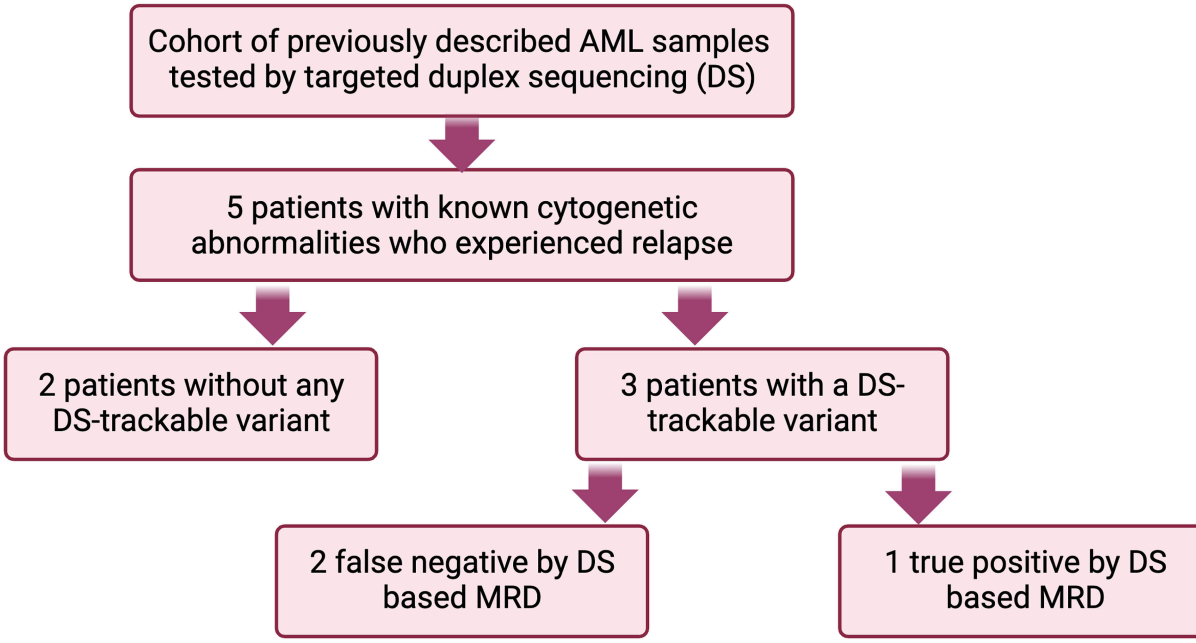
Abbreviations: WGS, whole genome sequencing; ddPCR, digital droplet PCR; MRD, measurable residual disease; MFC, multiparametric flow cytometry; DS, duplex sequencing; VAF, variant allele frequency; qRT-PCR, quantitative reverse transcription PCR; NCN, normalized copy number; ITD, internal tandem duplication

Figure Legends

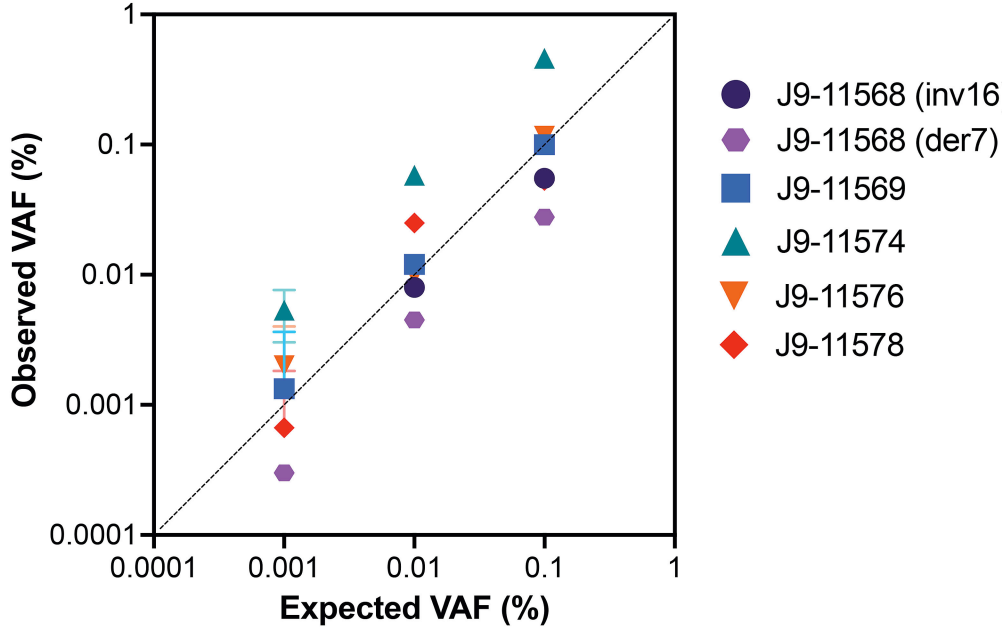
Figure 1. Assessment of WGS-informed patient personalized ddPCR assays.
(A) Outline of the study design. (B) Expected versus observed variant allele fraction (VAF) of patient-personalized whole-genome sequencing-informed ddPCR assays. Baseline genomic DNA or PCR amplicons (when baseline DNA was limiting) were diluted into healthy donor to simulate VAFs ranging from 0.1% to 0.0001%. Three replicates of 300ng/well or five replicates of 200ng/well for a total of 900-1000ng DNA of each dilution were run along with an adequate number of negative controls to calculate the limit of detection for each assay, which ranged from 0.01%-0.001%.

Figure 1

A

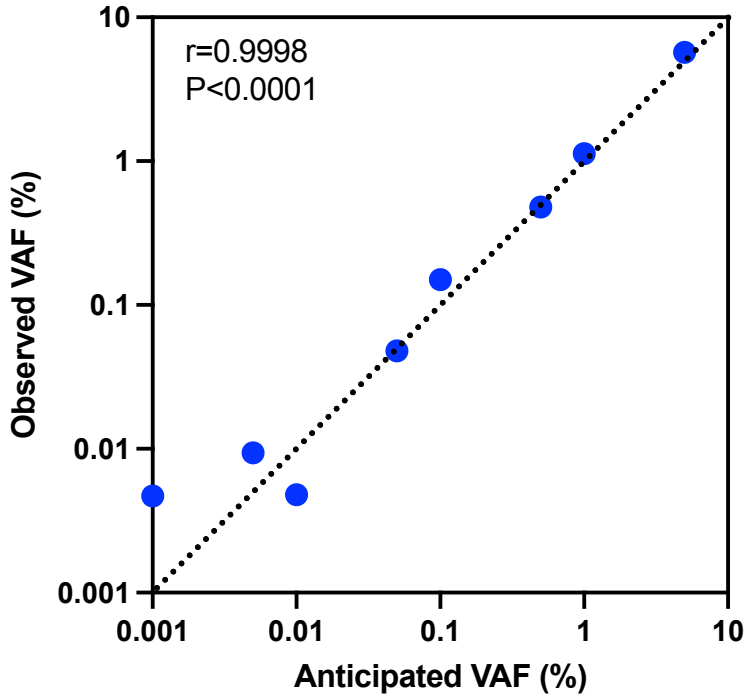

Cohort of previously described AML samples tested by targeted duplex sequencing (DS)
5 patients with known cytogenetic abnormalities who experienced relapse
2 patients without any DS-trackable variant
3 patients with a DS-trackable variant
2 false negative by DS based MRD
1 true positive by DS based MRD

B


Observed VAF (%)
Expected VAF (%)
1
0.1
0.01
0.001
0.0001
J9-11568 (inv16)
J9-11568 (der7)
J9-11569
J9-11574
J9-11576
J9-11578

Supplementary data

Supplementary Figure 1. Serial dilution of ME-1 cell line.
DNA from ME-1 cell line was diluted into DNA from a healthy donor at an expected variant allele fraction (VAF) of 5%-0.001% and the level of *CBFB::MYH11* was assessed at the DNA level using an ddPCR assay targeting the DNA breakpoints. 300-900ng (300ng/reaction) of DNA was tested at each dilution point and wells merged for the analyses. Wells with fewer than 10,000 droplets were excluded from the analysis. Thresholds separating positive and negative droplets were set using negative controls. The anticipated versus observed VAF is plotted with a line of equivalence plotted as a dashed line. The Pearson correlation and p-value are reported on the inset of the graph.



Supplementary Table 1. Sanger sequencing validation of genomic DNA breakpoints identified by whole genome sequencing (WGS).

Patient	WGS breakpoint	Sanger sequencing confirmed DNA breakpoint sequence[1]
J9-11578	6:18226235/9:131153503	TAATTCATTCCAGACTCTCAGCCTGAAAACACTGAGAATGTTTGCATGCTAGTTTTCCACATCATATACAATATTATTAAAAT ACTCATTTGGAATAGAATTCCATATGGGTTAACCAGAGTACTGTTGGGATGGTTGTGGCTATTTGCACGTAGCAGATTTCCT GCTTTTATTCAAAGACAATATTACTGGATTTTAAAATCTGCTTTTAACATTATTTTTCCTTTTCACTATACATAGGTCTATGAAA ATTACAGGCATCCGCTCTGCATCCGAGCTACATGTTGTTCCTATGTGCCTTTCCACCTCTTTCTCTATTGACAAACTAAGAGGA AAGCATACAGCTTTAGGGAGACACTTATTCAGGAGGGAATAATTTTCTTTTTTCATGGTGCTAAGTAGATAATACTAGTTAGC ATGTATTGAGTGTTTACTATGGACAATGCACAGATGATGAAACCAAGATAACTGAAACTGCCCAGGTTTTACCACTAATAAAC CTTGAAACCTTGAACCTGGG
J9-11574	16:15721335/16:67085250	GAGTGGTGATAGGAATGAAAAAGGCCACCCGACCTCCCTCTGCTGGCCTCCCCGGCAGCACGCACCTGTCTCTGCAGTTGC CTCCTCTTCTCCTCATTCTGCTCGTCCCGGGCTTGGAGATCCCTTTCGAACTGGCCCTTGAGCGCCTGCATGTTGACTTCCAG CCGCAGTTTGGCGTCCTCCGTGGCTTGCAGCTCGTCCTCCAGCTCTTCCAGCTGCGTCTTCATCTCCTCCATCTGGGTCTCCA GGGCCCGCTTGGACTTCTCCAGCTCATGGACCTGCCGGCAGAGCGGGCAGCCCCATTCTATGAGGCTCAACTTCATGAAGA GATTGAGAAACCCACCGTGAGCGGCACCTCAGGAGATCAGGGAGGTGGCTTTGGCCTCCCACAGGATGCATGGCCGGGAC TCAAGATGACCCCTGAGAGTTCAGACCCCAGCCTTATCCTCGGACCCCCCAACTCAGACCCATCCTCGACTGCCATTCTCAGC CCCTCCCAGCCCCTGCACCAGTCCAAAAACCTCCTTCCATTTCCGATGATAGTTCGCTATGAAAAAGGCC**T**TTGCGGTGAGCC GACATTGTGCCACTGCACTCCAGCCTGGGCGACAGAATGAGACTCCATCACACACACACACAGAGACACACACAGAGACAC ACACACACACACACAATTTATTTCATACACTAACCACCAAATAGGCTTGA
J9-11576	8:92066838/21:34844063	TTGGGCTAAGGGAGATGGACCTCTCTTTTGGATGATTTCCTAACTTCTCATCACTTTGACAGTCAATTAAATAGATACAACAC CTAATAAAAATGTACATTAATTTGTTTCTGCCACTGAAAGCCATCTGAAACTGTATTAAATAAAAGCCATAATAGATTGTCCC CTGACAAAGATAAATCCAATTATACTTTGTCTGCAAAAAAACACAAATGGTTTATGTTTTAGTTAGGCTACAATGTAAGATAC AGCTAACCACAAGAAATAAGTGGGATAGAGGGGGAAAGAAAAAGTTAAGGTCTTATCTCAAGGAAACCCCCAGTTACAGC GTAACTGAAAAGTAACCAGAGAGTGGTAACCACCAGTGACATGGATTTGTTGATCAGAGTAGCTCCTGCCCAGGGACAGAC TCTAAAGAGAAGGCGACTTGCAGAA
J9-11568	16:67088240/16:15721461	TCTATTCCAGGCAAAACGGTTCTTAATCTCTCCTGGCAGATTTTATTTTTCAGCTTAAGCATCTTTACGGGTGGTGTGGAAATTA ATTTCCCAGCGCTAAGCTGTATTTGTGCAGGCCTCCTGGTTAGGAAACTGGTTTAAATACTCACTGTTTCTTCCATTAACCAAAT ATTTGAGGGAGAGCAAGTCAATTAATTTTTTGGAGACTCTCAGTTTTATCAACTGTAATAATGTTAGATTAGATGATGTTTGAA TCTCTAAAGATAATCTGAATCACAGAGGGGAAACAACATTTTTCTCCAATATTCTCTTTTTAGGAAAAAGAAGGTAAACTGCAA GAAAATAAGGTGACATTAGGAATTTTTTATGGATGGAAAAAAAAAGACCATTTCGGCTTTGAGCATTTTGTTGGTCCGCTCGAGT TCCTCTTTGGCTTCCAAGGCCTCTTCAAGGGCCCGAGCCAGGGACAGGGCCTTGGTTTCCTTCTCCCTGGCTTCTGCCTCAGCTC TGTCCCTCTCATCCGCGTATTTGGAAGAGATGTTTTTCTCCTCGGCTAACAACTACAACACAAGACCCAGAGGTGACTTCTAGGC ATATCCGGGGTCAGCGTCACTGAATTGTAAATACCGGGGGAAGCCCTGTGTCCTGCTGAATGTATTGAGGTGCAGGTGTAAGC AGTGTAGGTTAGCTATGGGAGTAATTTATATAATCATCTGGCGTTCTGACTTCTACATCAACCTTATGCAGAGCCA
J9-11568	7:108403332/11:99267776	AAAGACCATACGAGAGAGCATGTGAAAGGTGTCAGTCCCTTGAGTCATCTCAAACCGAACTTTCTACTTTACAGATCCAAACAGG GCAGCTGTGCAGCAAGCCCCTCCTCCTTAGAGTGTCTTGAGTGAGTTGCTGTTTCCTTTGGTCCAGAGTGCCCAAATTCATACAG ACTAAGATTACAGCACTCCATTTACTGCCATTCTTACAGCTACATTTCAGACTGACAGTTAATATTTCTCTAATCTTTTTGTAGGTCTT TTTGTTAGAAACAAGTGAAGCCTTTATTTTCACAACATCAATATTTCACCTTCTTAGATTGGGTCCATTATCCCAACCTGCTAAAAAC TTTTGACTTCCCAATCTTGTCCTTACGAAATATTCATTATGCCAGGCCCAGCAGTAGTGTATGGATTCATCTGTGAGTTCCAGATATT CATGGTATCCTTGTATTTCTCTTCTATCACTTATGCCAAACTTAGAGATCTCTCTTATTCCCTCCTTGTGCTCTCTCTCTTTCTCTATC AAGTAAACACACACACACACGCGCGCACACACACACACACACACACACACACACACACACACATTATACAAAGGATCAAGTCTTGTT TGGGTGCAAGTTGAACCAAGAGCATGGTGTCCACATGACAGAGGTATTTCTTATTTAGTTAAGGTATGTGGCAGTCCTCATTTTAA ATCATCACTCT
J9-11569	16:15721403/16:67088894	TTCATGAAGACGATTGAGAAACCCACCGTGAGCGGCACCTCAGGAGATCAGGGAGGTGGCTTTGGCCTCCCACAGGATGCAT GGCCGGGACTCAAGATGACCCCTGAGAGTTCAGACCCCAGCCTTATCCTCGGACCCCCCAACTCAGACCCATCCTCGACTGCCA TTCTCAGCCCCTCCCAGCCCCTGCACCAGTCCAAAAACCTCCTTCCATTTCCGATGATAGTTCGCTATGAAAAAGGCCAGGAGCT AGCCTCGCATGGACTGGTGAATAGCACAGAGGGTGGGCAGGCGAAACATGGACGAGAAAAACCCCCTTAGTAATCTTAACCTT ACACATAGATTAAATACTCACGAGCACCACTGTTACAAGTTTAGCATGGCAGGGCTGGAGGCCTCAGAAGTTATCCAAACAGTG GAGGACATCCCTAAACCTCTGTGCTGCTAGGATCTCAAGCTTCCACAGGCCGTTTACTGACTTGCTGCCACCCATGAGTTACCAC TGTTCTCTGGCCCTCCTCTCCAGTCAGGCATCAGGACCACCCACTAGAACCATTATTTTTCTTTTCTGCCTCAGCCTTTTCTATTCC AGGCAAAACGGTTCTTAATC

[1]Primers were designed flanking the intronic breakpoints of the chromosomal translocation. The sequence of interest was amplified using the Q5 high-fidelity PCR kit (New England Biolabs). The amplified product was purified using SPRISelect beads (Beckman Coulter). Sequencing reactions were set up with the BigDye Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific) and capillary electrophoresis was performed on the ABI Genetic Analyzer 3500 (Applied Biosystems).

Supplementary Table 2. Quantitative RT-PCR MRD assay detection of fusion gene expression.

Patient ID	Timepoint	RNA equivalent input (ng)	Target[1]	Average ABL1 copies	Average fusion copies	Fusion NCN
J9-11576	Diagnostic	100	*RUNX1::RUNX1T1*	7,642[3]	131,817	17.2494
J9-11568	Diagnostic	90[2]	*CBFB::MYH11*	12,688	29,681	2.3393
J9-11569	Diagnostic	32[2]	*CBFB::MYH11*	2,128[3]	7,513	3.5313
J9-11576	Remission	31[2]	*RUNX1::RUNX1T1*	0[3]	0	0
J9-11568	Remission	100	*CBFB::MYH11*	2,760[3]	5	0.0019
J9-11569	Remission	100	*CBFB::MYH11*	1,377[3]	7	0.0052
J9-11574	Remission	32[2]	*CBFB::MYH11*	671[3]	0	0

Abbreviations: NCN, normalized copy number

[1]Expression of the *RUNX1::RUNX1T1* or *CBFB::MYH11* RNA fusion transcript levels were detected using the *ipsogen* CBFB-MYH11 A (Qiagen cat# 676013) or RUNX1-RUNX1T1 (Qiagen cat# 675013) following manufacturer's instructions. In short, 500ng (or less if insufficient material available) of RNA extracted for time of diagnosis or remission bone marrow mononuclear cells was reverse transcribed into cDNA using the SuperScript III first-strand synthesis system (ThermoFisher Scientific, cat# 18080051). Resulting cDNA, along with included standard dilution controls and NTC, was amplified using primers and Taqman probes for the specific fusion transcript of interest and ABL control in duplicate and fluorescence detected using the QuantStudio 3 real-time PCR system (ThermoFisher Scientific). A standard curve for each gene was obtained from the plasmid serial dilutions and used to calculate the copy number for each sample.

[2]Insufficient RNA input (<100ng equivalent) available to achieve maximal assay sensitivity

[3]ABL1 copies less than recommended level of ≥10,000 (PMID:41397238)