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Letter
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ABL-class Genomic Breakpoint Q-PCR: A Patient-specific Approach for MRD Monitoring in Acute Lymphoblastic Leukemia

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ABL-class fusions, in this study defined as fusions of ABL-class kinases other than *BCR*::*ABL1*, have been identified in 3%–5% of newly diagnosed pediatric and adult acute lymphoblastic leukemia (ALL).[1–3] These fusions comprise driver genes *ABL1, ABL2, PDGFRB*, or *CSF1R* fused to a wide range of partner genes. In B-cell ALL (B-ALL), *PDGFRB* fusions are the most common, whereas *ABL1* fusions are the most common in T-cell ALL (T-ALL).[1–3] Generally, ABL-class B-ALL patients respond poorly to standard induction therapy.[1] Therefore, these patients receive targeted therapy with tyrosine kinase inhibitors additional to chemotherapy.[4] Accurate monitoring of measurable residual disease (MRD) is critical to evaluate treatment response allowing proper risk stratification and early detection of refractory or relapsed ALL.

Determination of MRD by quantitative polymerase chain reaction (Q-PCR) for ALL patients is highly standardized using 2 immunoglobulin and T-cell receptor (IG/TR) rearrangements selected at diagnosis. However, there are some limitations; in particular the lack of identifiable or sensitive IG/TR targets for about 5% of patients[5–8] and the loss of the selected IG/TR gene rearrangement due to ongoing rearrangements and/or oligoclonality.[7,9,10] As an alternative to the IG/TR method, fusions often act as primary drivers of ALL that allow for MRD determination by PCR using the fusion transcript or the genomic breakpoint. High correlations between the IG/TR MRD and genomic breakpoint MRD have been shown in multiple studies with various B-ALL subtypes.[11–13] In a large study focusing on *BCR*::*ABL1* B-ALL patients, discordant MRD results between IG/TR PCR and fusion transcript or genomic breakpoint PCR were found for ≈25% of patients. This led to the identification of chronic myeloid leukemia (CML)-like disease, characterized by presence of the *BCR*::*ABL1* fusion gene in blood cell lineages other than B-cell in patients presenting with B-ALL.[12,14] MRD discordance is caused by eradication of the ALL cells containing the IG/TR target during treatment but remaining *BCR*::*ABL1* positivity from the other cell types.[14] Considering the similarity to *BCR*::*ABL1* B-ALL, a disease involving hematopoietic lineages other than the B-cell similar to CML-like disease could possibly also occur in ABL-class ALL.[12]

In this retrospective study, we aimed to assess the technical and clinical advantages of genomic breakpoint-based MRD analysis compared with conventional IG/TR-based MRD analysis using patient-specific Q-PCR assays directed against ABL-class genomic breakpoints. We showed that the genomic breakpoint approach allowed monitoring of MRD in all patients with good quality DNA of follow-up time points available, including those without suitable IG/TR targets, and overcame the underestimation of the MRD levels due to oligoclonality. Moreover, our results demonstrated that the combination of the conventional IG/TR approach and genomic breakpoint approach is recommended for early detection of involvement of non-B-cell hematopoietic lineages.

We used targeted sequencing approaches on leftover DNA from diagnosis to determine the genomic breakpoints for 22 ABL-class ALL, including 18 with B-ALL and 4 with T-ALL (Suppl. Table S1). *ABL1* had the largest genomic breakpoint spanning region of ≈150 kb, extending into the 3′ untranslated region of *EXOSC2* in a *ZMIZ1*::*ABL1* patient (pat #16) (Suppl. Table S1; Suppl. Figure S1). The long-read based method fusion detection from gene enrichment identified the genomic breakpoint by covering the entire gene and a ≥10,000 bp region from the target site (Suppl. Table S2), while the genomic breakpoint could not be detected by genomic capture high-throughput sequencing (gc-HTS), which covers the specified genes but not the upstream regions. *PDGFRB* had a smaller breakpoint spanning region of about 5.3 kb, suitable for detection with long-range PCR and standardized primers (Suppl. Table S1;

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Suppl. Figure S1). The breakpoint spanning regions for *ABL2* and *CSF1R* fused samples could not be identified due to limited sample numbers, but the predicted regions are ≈4.5 and 6.3 kb, respectively. Therefore, long-range PCR is likely to be effective in identifying these fusions as well (Suppl. Table S1; Suppl. Figure S1).

Patient-specific Q-PCR MRD assays were developed using genomic breakpoints. A quantitative range of ≤1E-4 was observed for 72% of the genomic breakpoint assays and 100% of the IG/TR assays and all assays had a sensitivity of ≤1E-4, similar to that described previously for other patient-specific fusions and deletions (Figure 1A and 1B).[11] The genomic breakpoint assays with a quantitative range of 5E-4 exhibited less reproducible amplification (Suppl. Table S3). The enhanced quantitative range of IG/TR assays may be attributed to selection of the most optimal target, while genomic breakpoint assays are limited to a single target option, leaving limited options for optimal assay design.

Genomic breakpoint MRD levels were determined for 21 ABL-class ALL patients with good quality DNA for at least 1 follow-up time point, while IG/TR MRD data was available for 19 patients; 2 B-ALL patients (pat #2 and #10) and 1 T-ALL patient (pat #14) lacked suitable IG/TR targets at diagnosis. Notably, the leukemic clones in these 3 patients could effectively be followed using the genomic breakpoint (Suppl. Figure S2). The MRD levels of 18 patients (72 samples) who had both IG/TR and patient-specific genomic breakpoint Q-PCR results for follow-up time points were highly correlated with a Spearman correlation coefficient of 0.97 ($P < 0.001$; Figure 2A), with high concordance observed at important time points for risk stratification (91% at end of induction (EOI) and 93% at end of consolidation ([EOC]; Figure 2B and 2C).

Outside the quantifiable range, we observed discrepant results in 5 samples from 3 individual patients (pat #4, #13, and #19) that were negative by IG/TR and positive but not quantifiable by genomic breakpoint Q-PCR or vice versa (Figure 2A). Additionally, the MRD level of 1 sample (pat #6) could be quantified by the genomic breakpoint approach due to an outstanding quantitative range (QR 1E-5) and was positive but not quantifiable by the IG/TR approach (QR 1E-4) (Figure 2A; Suppl. Figure S2).

Despite of the high overall correlation between the genomic breakpoint MRD and the IG/TR MRD, we identified 2 cases with discordant results within the quantitative range. Patient 19, with *NUP214*::*ABL1* fusion, had a >10-fold higher MRD after induction therapy based on the genomic breakpoint (0.4%) compared with the IG/TR target (0.03%) (Figure 2A, 2B, and 2D). This was explained by a subclone of leukemic cells that was not captured by IG/TR Q-PCR and was confirmed by flowcytometric MRD analysis (Figure 2D). Another patient (pat #12), with a *CCDC88C*::*PDGFRB* fusion, consistently had higher genomic breakpoint levels (2%–20%) compared with the IG/TR levels (pos, NQ/negative) from EOC onwards (Figure 2A, 2C, and 2E). Morphological data of EOI (M1), hampered by a substantial number of dead cells, showed 6% blasts, confirming the IG/TR results measuring 50% blasts. No blasts were identified at EOC (M3) or at a follow-up time point (M11) by morphological evaluation or IG/TR MRD, while genomic breakpoint PCR showed 20% fusion positivity (Figure 2E). The presence of the fusion gene combined with the absence of blasts suggests involvement of non-B-cell hematopoietic lineages in this ABL-class ALL patient, similar to CML-like disease described in *BCR*::*ABL1* B-ALL.

In this study, we assessed the technical feasibility and clinical advantages of MRD determination using the genomic breakpoint of ABL-class ALL patients. We successfully identified the genomic breakpoints for all ABL-class ALL patients. Surprisingly, 1 *ABL1*-fused patient harbored a genomic breakpoint upstream of *ABL1*, which was not detected with gc-HTS, a targeted approach restricted to the *ABL1* region. Although upstream genomic breakpoints are not uncommon and have been reported in CML,[15–17] this is the first ABL-class ALL reported with an upstream genomic breakpoint. This case highlights the importance of the use of a technique with a broad target area. While rapid genomic breakpoint detection is possible using long-range PCR for cases with small breakpoint spanning regions (<20,000 kb), it remains hard for large breakpoint spanning regions hindering rapid development of genomic breakpoint MRD assays. However, well-designed capture panels or implementation of whole genome sequencing will allow for

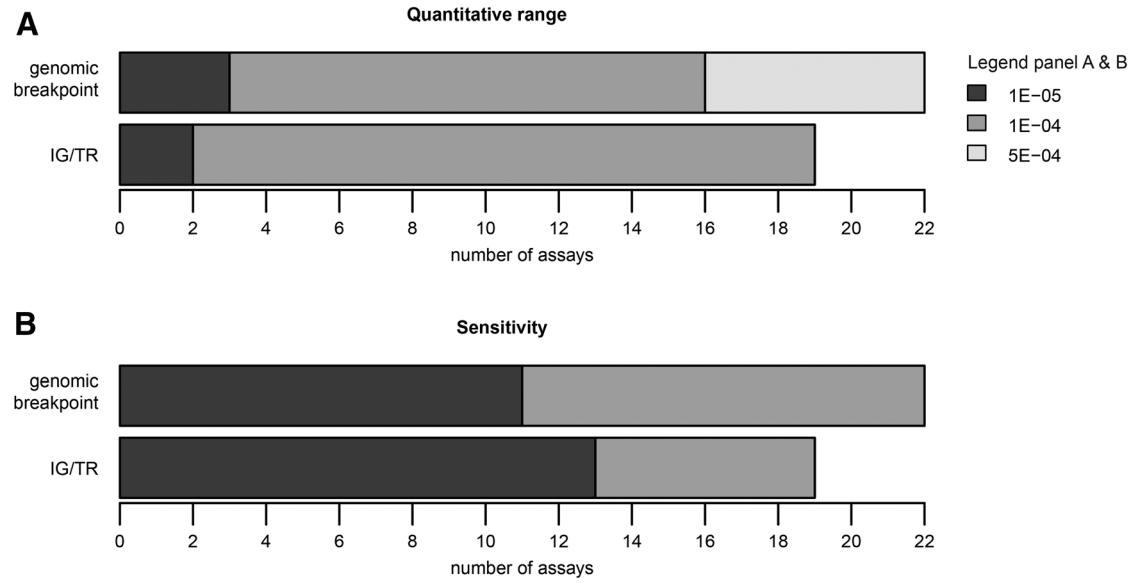

Figure 1. Performance of the IG/TR and the genomic breakpoint Q-PCR assays. (A) Evaluation of the quantitative range of the genomic breakpoint Q-PCR assay (n = 22) and IG/TR Q-PCR assay of the target used for the clinically reported MRD (n = 19). (B) Evaluation of the sensitivity of the genomic breakpoint Q-PCR assay (n = 22) and IG/TR Q-PCR assay of the target used for the clinically reported MRD (n=19). In case of different quantitative ranges or sensitivities at various time points for IG/TR MRD determination, the first measurement was used. IG/TR = immunoglobulin and T-cell receptor; MRD = measurable residual disease.

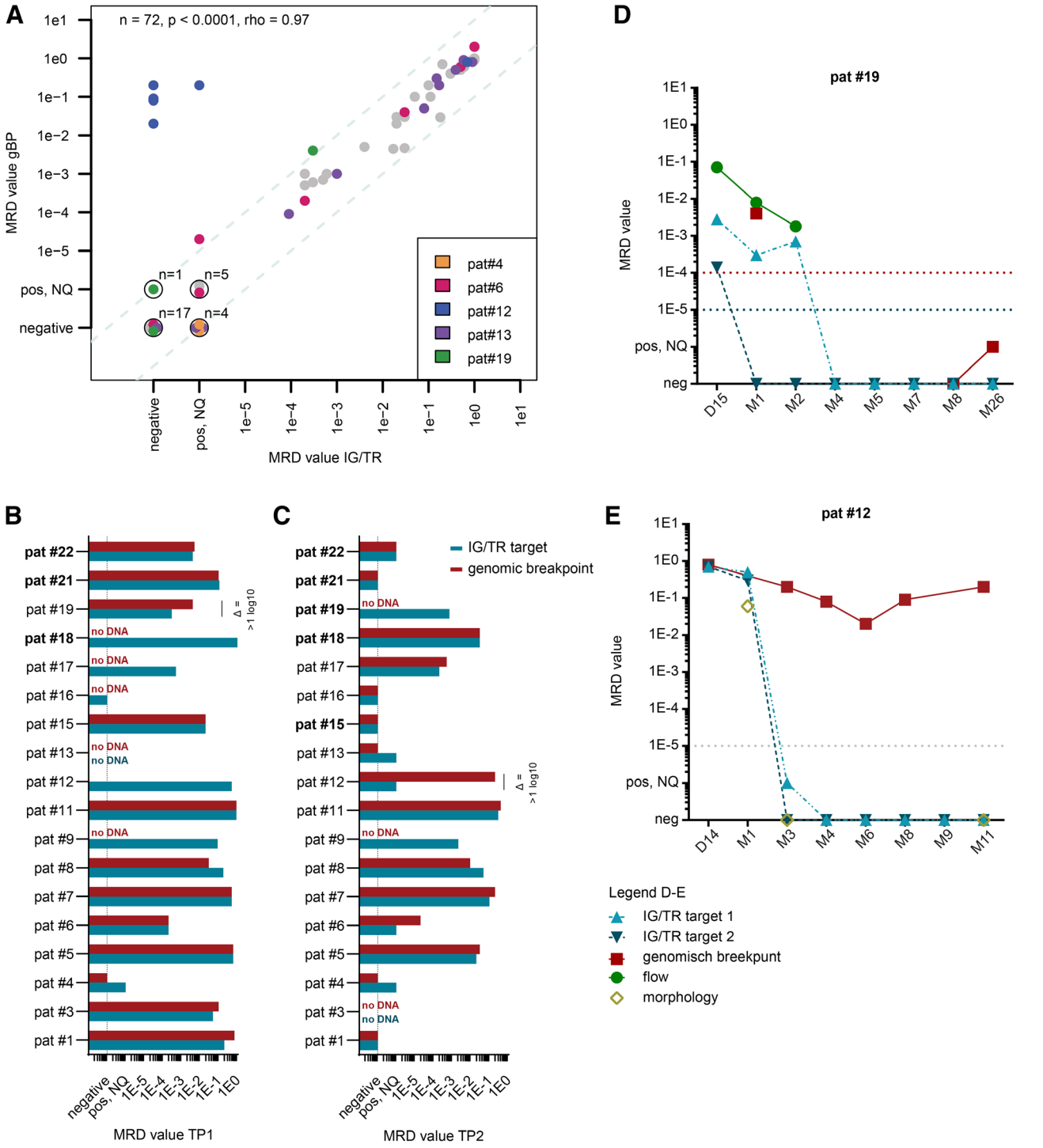

Figure 2. Comparison between IG/TR MRD and genomic breakpoint MRD. (A) Comparison between clinically reported MRD based on IG/TR Q-PCR and genomic breakpoint Q-PCR MRD (n = 72 samples from 18 patients). Patients with discordant MRD results >1 log10 difference (beyond the dashed lines) at least studied follow-up time point were visually distinguished by assigning them a color. In case of pos, NQ or negative MRD levels, multiple samples have the same coordinates in the plot, and the number of samples at the location specified by a circle are depicted at the top right of the circle. (B) Comparison of the clinically reported MRD levels by IG/TR Q-PCR and the genomic breakpoint MRD at EOI, defined between day 29 and day 43. If there were multiple measurements within this time frame, the first one was taken. (C) Comparison of the clinically reported MRD levels by IG/TR Q-PCR and the genomic breakpoint MRD at EOC defined between day 71 and day 115. If there were multiple measurements within this time frame, the first one was taken. (B and C) Discordant levels (>1 log10 difference) are highlighted by a bar; patients treated with a tyrosine kinase inhibitor before the corresponding time point are bold; vertical dotted line represents a negative MRD level; in case of missing values, this is denoted by "no DNA" meaning there was no or not sufficient follow-up DNA. (D) Kinetics of patient 19 harboring a subclone that was not detected by the IG/TR Q-PCR. MRD levels based on IG/TR (triangles) compared with genomic breakpoint (squares) and flow MRD (circles). (E) Kinetics of patient 12 with involvement of non-B-cell hematopoietic lineages. MRD levels based on IG/TR (triangles), genomic breakpoint (squares), and morphology (triangles). The horizontal dotted lines represent the sensitivity of the assay: genomic breakpoint assay in red, the most sensitive IG/TR assay in turquoise, in case of the same sensitivity for the genomic breakpoint and the most sensitive IG/TR assay in gray. D = day; EOC = end of consolidation; EOI = end of induction; IG/TR = immunoglobulin and T-cell receptor; M = month; MRD = measurable residual disease; Neg = negative; Pos, NQ = positive but below quantitative range of the assay; Q-PCR = quantitative polymerase chain reaction.

genomic breakpoint identification even in these large breakpoint spanning regions.[18,19]

Our study showed that while there was a high correlation between IG/TR MRD levels and genomic breakpoint MRD levels, the genomic breakpoint approach overcame certain limitations of the IG/TR approach. Previous studies described inaccuracies in determining the IG/TR levels due to outgrowth of subclones lacking the selected IG/TR rearrangements at diagnosis resulting in false-negative results.[7,9] We identified 1 patient with discordant MRD results between IG/TR and genomic breakpoint levels due to the selection of subclonal IG/TR targets at diagnosis, highlighting the risk of underestimating MRD levels and potentially leading to suboptimal treatment decisions when relying solely on IG/TR assays. In contrast, many fusion genes are the drivers of leukemia; hence, they are reliable genetic markers during disease progression. Additionally, around 5% of patients lack suitable IG/TR targets at diagnosis.[5–8] Our cohort contained 3 patients (14%; 2 B-ALL and 1 T-ALL) for whom with conventional IG/TR methods no targets could be identified, but on the genomic breakpoint level the leukemic cells could be traced.

In conjunction with the overall constraints of IG/TR MRD measurement it is important to consider the presence of the fusion gene in hematopoietic cell lineages other than B-cells, like shown in *BCR*::*ABL1* B-ALL with CML-like disease.[14] We identified 1 ABL-class ALL patient with high genomic breakpoint MRD levels and negative IG/TR levels, suggesting involvement of non-B-cell hematopoietic lineages. Detecting involvement of other hematopoietic lineages is important, since in the ALLTogether protocol, currently used for (pediatric) ABL-class patients, MRD levels at the end of induction are used for risk stratification, while in CML-like disease both IG/TR and genomic breakpoint MRD show limited prognostic relevance.[20] Venn et al studied genomic breakpoint MRD in a similar sized cohort of ABL-class ALL patients and did not identify involvement of non-B-cell hematopoietic lineages in any of the patients.[12] This implies that, unlike the occurrence of CML-like disease in 25% of pediatric *BCR*::*ABL1*-positive B-ALL,[20] involvement of blood cell lineages different from the B-cells in ABL-class ALL is rare.

In conclusion, we demonstrate that it is technically feasible to develop sensitive genomic breakpoint Q-PCR assays for ABL-class patients that show high concordance with IG/TR Q-PCR MRD. Furthermore, we demonstrate that using genomic breakpoint Q-PCR is of clinical relevance because it provides a higher level of certainty in tracking the entire leukemic clone and can detect involvement of blood cell lineages different from the B-cells. Therefore, we recommend using the genomic breakpoint for MRD determination in conjunction with IG/TR methods for ABL-class ALL.

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AUTHOR CONTRIBUTIONS

This project was conceived by MLdB and conceptualized together with JMB and IvO. IvO, VHJvdV, PGH, ES, and UzS designed quantitative polymerase chain reaction (Q-PCR) assays, performed measurable residual disease (MRD) tests, and analyzed the results. UzS, GvH, IvO, and RPK performed genomic capture high throughput sequencing, fusion detection by gene enrichment, and targeted locus amplification, and the corresponding genomic breakpoint analysis. UzS, GE, VHJvdV, PGH, ES, and GEV, provided samples and data. The article was drafted by IvO, JMB, and MLdB. All authors reviewed and approved the article.

DISCLOSURES

The authors have no conflicts of interest to disclose.

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