blood advances

TO THE EDITOR:

Minimal disease detection by immunoglobulin high-throughput sequencing in pediatric Burkitt lymphoma

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Burkitt lymphoma (BL) is the most common pediatric non-Hodgkin lymphoma and has a favorable overall survival, but survival after relapse is poor, and therapy is fraught with toxicity. BL clinical staging and response evaluation currently rely on imaging (ie, computed tomography and positron emission tomography), bone marrow (BM) evaluation, and cerebrospinal fluid evaluation. Apart from recent data showing that somatic *TP53* mutations are prognostic in pediatric BL,[1-4] molecular techniques for enhancing staging, monitoring treatment response, and guiding therapy are lacking. For example, stage IV BL, defined by pretreatment BM or central nervous system involvement,[5] is associated with treatment resistance and inferior outcomes, but some patients without clinically apparent BM involvement still relapse. Retrospective analyses have supported the prognostic value of minimal marrow disease (MMD) or minimal disseminated disease (MDD) detected via long-distance polymerase chain reaction (PCR) of the BL-associated t(8;14) translocation,[6] albeit with some inconsistency in the rituximab era,[3] but this has not been definitively implemented for guiding BL treatment. As a result, focused trial initiatives to deintensify toxic therapy have been precluded by an inability to identify patients with the most favorable prognosis.

High-throughput sequencing (HTS) of rearranged immunoglobulin gene loci has enhanced the sensitivity of minimal residual disease (MRD) detection for treatment response evaluation and surveillance of other B-cell cancers, including B-lymphoblastic leukemia[7,8] and adult diffuse large B-cell lymphoma.[9] However, this clinically available assay has not been implemented for BL despite confirmation of clonal immunoglobulin gene rearrangements in BL tumor tissue and preliminary immunoglobulin PCR MDD data.[10,11] As a critical step toward testing the clinical immunoglobulin HTS assay in BL, we sequenced tumor-associated immunoglobulin gene rearrangements in paired BM and peripheral blood (PB) samples to test the feasibility of this technique for MMD/MDD detection and treatment response evaluation. Using genomic DNA (gDNA) extracted from residual diagnostic tumor samples from 11 patients with sporadic BL diagnosed and treated at our single academic medical center (supplemental Table 1), we performed immunoglobulin heavy chain (*IgH*) sequencing using targeted primers (supplemental Table 2) according to established methods.[12] We defined clonal *IgH* rearrangements by their complementarity determining region 3 nucleotide sequences comprising $\geq$5% of total *IgH* sequencing reads (supplemental Methods), revealing 1 to 2 per tumor, with a median abundance of 50.1% (range, 8.4-90.5; Figure 1A). Having confirmed our ability to accurately discern relative clone abundance using this technique (supplemental Figure 1), we evaluated pretreatment staging BM samples from 8 patients with evaluable BM to qualitatively test the feasibility of *IgH* sequencing for MMD detection. We validated our findings in 2 patients using the clinical immunoglobulin HTS clonality identification (ID) and tracking assays (ClonoSEQ; Adaptive Biotechnologies). We also applied targeted *TP53* sequencing as an independent, clinically relevant variant for MMD validation. Finally, we tested the clinical immunoglobulin HTS assay (ClonoSEQ) on 5 serial PB

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Immunoglobulin heavy chain next-generation sequencing data are available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1086378.

The full-text version of this article contains a data supplement.

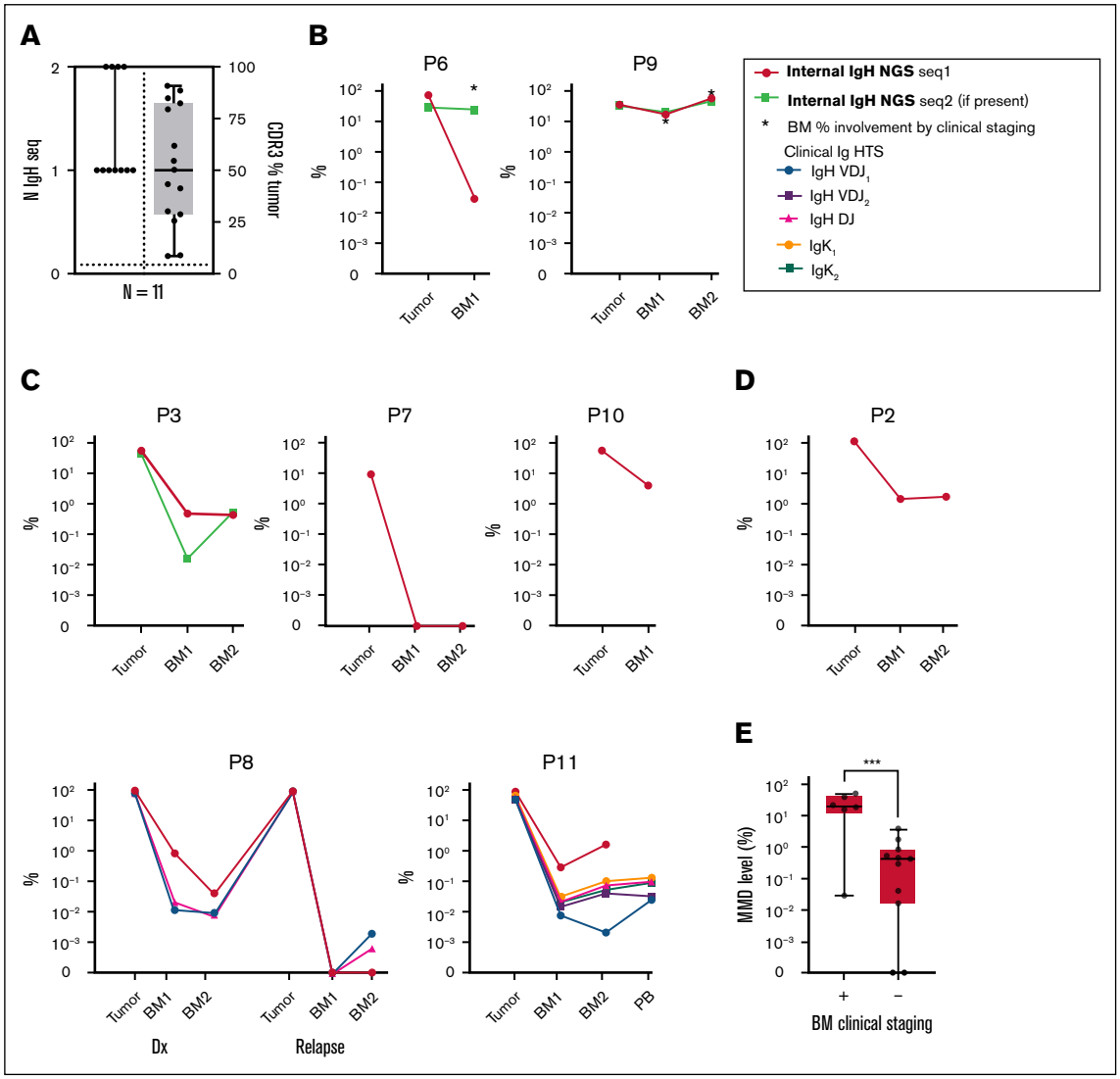

Figure 1. MMD detection via immunoglobulin HTS in BL. (A) The total number of tumor-associated, clonal *IgH* sequences per patient (N IgH seq) and their relative abundance as a proportion of total *IgH* sequencing reads in each tumor sample (CDR3% tumor) are shown for 11 patients (P1-P11). (B-D) Relative clonal sequence abundance between tumor biopsy and staging BM samples is shown for patients with positive BM clinical staging (n = 2; B), negative BM clinical staging (n = 5; C), and "indeterminate" clinical staging (n = 1; D). Clinical immunoglobulin HTS data (ClonoSEQ) pertaining to the abundance of tumor-associated immunoglobulin sequences among total nucleated cells assayed are incorporated for 2 patients (P8 and P11). "BM1" and "BM2" labels indicate bilateral BM samples obtained simultaneously for clinical staging, when available. (E) MMD level (%) of each clonal *IgH* sequence in clinically positive vs negative BM samples (mean, 24.5% vs 0.746%; *P* = .005). CDR3%, complementarity determining region 3 sequence percent; Dx, diagnosis; NGS, next-generation sequencing.

samples from a single patient to establish the feasibility of HTS-based treatment response evaluation in BL.

Immunoglobulin HTS detected more BL MMD than conventional standard-of-care (SOC) methods, including flow cytometry. Eight patients had staging BM samples available for evaluation: 6 bilateral (obtained simultaneously) and 2 unilateral. We detected tumor-associated *IgH* rearrangements in the BM of all 3 patients with either "positive" or "indeterminate" BM staging as well as in 4 of the 5 patients negative for BM involvement by SOC methods (Figure 1B-D). Mean MMD level was greater in samples from patients with BM involvement by SOC than those without (24.5% ± 18.2% vs 0.746% ± 1.15%; *P* = .0005; Figure 1E). Furthermore, 1 patient reported negative for BM involvement (P8) went on to experience tumor progression/relapse. The same clonal rearrangement in the diagnostic tumor sample recurred in the relapse tumor (Figure 1C). The clinical immunoglobulin HTS assay (ClonoSEQ) confirmed that the same tumor-associated *IgH* variable-diversity-joining (VDJ) sequence (supplemental Table 3) was present at both time points as well as an additional trackable, incomplete DJ rearrangement. Both sequences confirmed MMD at initial diagnosis and persistence in 1 of 2 restaging BM samples at relapse (Figure 1C).

Because of the prevalence and prognostic relevance of *TP53* mutations in pediatric BL, we also applied targeted *TP53*

sequencing via established methods[13] to extracted gDNA from all 11 BL tumor samples and paired staging BM samples from 8 patients. We discovered heterozygous, damaging *TP53* mutations in tumor samples from 2 patients: P2 had 2 damaging mutations, with variant allele frequencies (VAF) of 0.45 and 0.44; P8 had 2 damaging mutations, with VAF of 0.49 and 0.48. In both patients, one or both mutations were also detectable at low VAF in corresponding BM sample(s) (supplemental Table 4). At relapse, the *TP53* mutations from the P8 diagnostic tumor were detected at VAF of 0.49 and 0.47, respectively, in the relapse tumor and again in the corresponding BM (supplemental Table 4).

Finally, to further explore the feasibility of immunoglobulin HTS for treatment response evaluation and surveillance, we tested the clinical immunoglobulin HTS assay (ClonoSEQ) using 2 specimen sources from a single patient (P11) collected across 5 serial time points: gDNA extracted from PB cells and circulating tumor DNA (ctDNA) from PB plasma. The clinical assay confirmed our previously defined tumor-associated clonal *IgH* VDJ rearrangement (supplemental Table 3) as well as an additional trackable *IgH* VDJ, an incomplete DJ, and 2 immunoglobulin kappa rearrangements with relative concordance in MMD detection across bilateral staging BM samples (Figure 1C). Immunoglobulin HTS confirmed MDD in the pretreatment PB (Figures 1C and 2A), followed by serial attenuation using the cellular assay (Figure 2A). In contrast, rising plasma ctDNA across early treatment time points (Figure 2A) resulted in higher detection than the cellular assay after 28 days (Figure 2B).

Our data reveal clonal *IgH* gene rearrangements in all BL tumor samples and more sensitive MMD detection via immunoglobulin HTS than via conventional SOC. These findings suggest that immunoglobulin HTS could have practice-changing implications for improving BL risk stratification, particularly if patients with undetectable MMD prove to have a better prognosis; our data offer critical justification for testing prognostic value in the context of trial-associated collections. Comparable MMD detection across bilateral BM samples (supplemental Figure 2) as well as concurrent *TP53* mutation detection in the BM of 2 patients whose tumors harbored mutations (supplemental Table 4) highlight the reliability and reproducibility of immunoglobulin HTS as a measure of systemic BL dissemination. Furthermore, our data reveal that treatment response evaluation via the clinical cellular immunoglobulin HTS minimal residual disease assay is feasible, whereas the ctDNA assay, which instead showed rising levels potentially indicative of treatment-induced tumor cell apoptosis (Figure 2), might be most appropriate for exploration as a surveillance tool after complete disease clearance.

Immunoglobulin HTS as a measure of treatment response and disease surveillance is a well-studied clinical tool for other B-cell–derived cancers[7,9,14-17] but has not been implemented for pediatric BL. Despite ample feasibility data in other non-Hodgkin lymphomas[9,18-20] and long-standing evidence that BL MMD/MDD detection is prognostic by other methods, such as PCR targeting of hallmark BL chromosome 8 translocations and/or break points involving the *cMYC* gene,[6,21-25] the prognostic utility of the broadly clinically available immunoglobulin HTS assay has not been systematically evaluated in BL. Although our study's small sample size and lack of annotated clinical data precluded our ability to link MMD with outcomes, our findings nevertheless confirm the feasibility and sensitivity of HTS MMD/MDD detection in BL and provide critical justification for larger, prognostic analyses among clinically annotated, trial-associated collections.

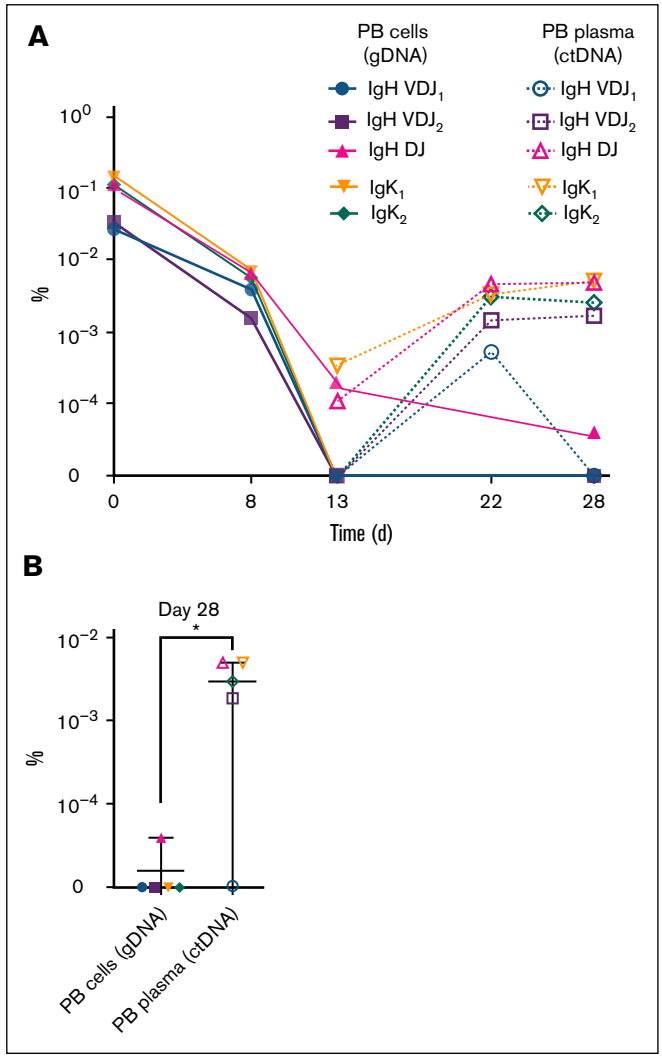

Figure 2. MDD tracking via immunoglobulin HTS in BL. (A) Clonal sequence abundance detected by clinical immunoglobulin HTS (ClonoSEQ) is shown across 5 serial PB samples collected from 1 patient (P11). Data from gDNA extracted from PB cells are available from treatment days 0, 8, 13, and 28; data from ctDNA extracted from PB plasma are available from days 13, 22, and 28. (B) Discordant MDD detection between PB cells vs plasma (ctDNA) on treatment day 28 ($P = .015$); all trackable sequences are shown.

In summary, immunoglobulin HTS may have a practice-changing clinical role for enhancing detection and surveillance of BL MMD and MDD and warrants evaluation in the trial setting. The broad accessibility of this assay makes it an appealing technique for evaluation in this in-need patient population.

Data presented in this manuscript were generated in accordance with a University of Rochester Institutional Review Board–approved protocol. This study was conducted in accordance with the Declaration of Helsinki.

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