STIMULUS REPORT

blood advances

VpreB surrogate light chain expression in B-lineage ALL: a report from the Children's Oncology Group

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Key Points

- The VpreB component of the SLC is expressed in standard- and high-risk B-ALL.
- The VpreB may serve as a novel immunotherapeutic target.

Immunotherapies directed against B-cell surface markers have been a common developmental strategy to treat B-cell malignancies. The immunoglobulin heavy chain surrogate light chain (SLC), comprising the VpreB1 (CD179a) and Lamda5 (CD179b) subunits, is expressed on pro- and pre-B cells, where it governs pre–B-cell receptor (BCR)-mediated autonomous survival signaling. We hypothesized that the pre-BCR might merit the development of targeted immunotherapies to decouple "autonomous" signaling in B-lineage acute lymphoblastic leukemia (B-ALL). We used the Children's Oncology Group (COG) minimal residual disease (MRD) flow panel to assess pre-BCR expression in 36 primary patient samples accrued to COG standard- and high-risk B-ALL studies through AALL03B1. We also assessed CD179a expression in 16 cases with day 29 end-induction samples, preselected to have ≥1% MRD. All analyses were performed on a 6-color Becton-Dickinson flow cytometer in a Clinical Laboratory Improvement Amendment/College of American Pathologist–certified laboratory. Among 36 cases tested, 32 cases were at the pre-B and 4 cases were at the pro-B stages of developmental arrest. One or both monoclonal antibodies (mAbs) showed that CD179a was present in ≥20% of the B-lymphoblast population. All cases expressed CD179a in the end-induction B-lymphoblast population. The CD179a component of the SLC is commonly expressed in B-ALL, regardless of genotype, stage of developmental arrest, or National Cancer Institute risk status.

Introduction

The productively assembled pre–B-cell receptor (pre-BCR) autonomously signals to govern immature B-cell selection and expansion into immunoglobulin-producing cells.[1] The pre-BCR is composed of 5 units (see visual abstract): a membrane-bound V-, D-, J-recombined immunoglobulin heavy chain, an invariably constant surrogate light chain (SLC), comprising VpreB (CD179a) and λ5 (CD179b),[1] and transmembrane immunoglobulin α (Igα) and Igβ accessory chains that coassemble to provide intracellular signaling through SRC and SYK family kinases.[2,3] Differentiation into mature B cells can only occur when immature B-precursors have undergone recombination of genes encoding κ or λ light chains, which dynamically replace the SLC in

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The authors are happy to share raw data from flow experiments with qualified experts: e-mail the corresponding author at stuart.winter@childrensmn.org for requests. The annotated clinical data are available through the Children's Oncology Group Cell Bank and its standardized approval processes.

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

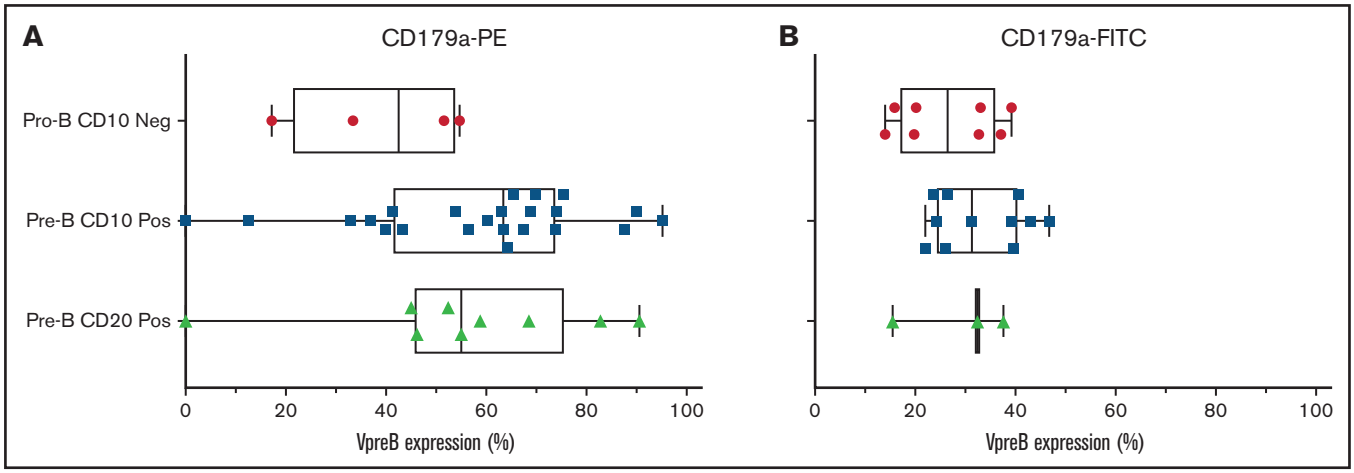

Figure 1. Using the VpreB-PE and the VpreB-FITC mAbs, 36 diagnostic cases were tested for VpreB surface expression in day 0 cryopreserved samples that were obtained from children and young adults with NCI standard- and high-risk B-ALL. Cases were subdivided into pro-B and pre-B-ALL based upon the absence or presence of coexpression with CD10 and CD20. There were no statistical differences in VpreB expression among these 3 subgroups, but all cases except 4 showed >20% expression using either the PE-conjugated (A) or the FITC-conjugated (B) CD179a mAbs. Lack of VpreB expression could not be correlated with the presence or absence of any recurring molecular aberrations, as shown in supplemental Table 2. Neg, negative; Pos, positive.

maturing B cells to create a functional BCR.[3] Without pre-BCR mediated "tonic" autonomous signaling, immature B cells undergo programmed cell death, but this critical selection step may be subverted by oncogenic transformation.[1,4]

Despite numerous genomic aberrations, nearly all B-lineage acute lymphoblastic leukemia (B-ALL) cases share a relatively restricted repertoire of B-cell surface markers, including CD19 and CD22, and with variable expression of CD34 or CD20.[5-7] Despite the use of risk-adjusted therapies, relapse is a common problem for infants, adolescents, and adults.[5,8] Novel immunotherapies have the potential to uncover unexpected escape pathways by which leukemic cells evade cell death.[9] Although relatively little is known about the expression of the pre-BCR in B-ALL, others have concluded that the pre-BCR is functionally active in a small but important subset of ~16% cases, designated "pre-BCR+ ALL."[7,10,11] For antibody-mediated therapy, surface expression, not signaling, mediates cell killing, as demonstrated by the efficacy of rituximab against many CD20-expressing neoplasms, including B-ALL.[6]

We recently described the characteristics of a novel high-affinity, high-avidity anti–pre-BCR antibody and evaluated whether blockade of homotypic pre-BCR self-associations might differentially sensitize primary patient samples to chemotherapy.[2] We found that incubation of patient blasts with anti-VpreB monoclonal antibodies (mAbs) enhanced apoptosis by decoupling cell survival pathways. Because B-ALLs might resist cytotoxic therapies by means of autonomous survival signaling, we investigated whether CD179a, as an immunotherapeutic target, might be more commonly expressed on B-lymphoblasts than previously reported.[10]

Methods

To assess CD179a surface expression in B-ALL, we used National Cancer Institute (NCI) risk status and end-induction minimal residual disease (MRD) levels of ≥1% to select 36 diagnostic cases from Children's Oncology Group (COG) Biology Study AALL03B1 (#NCT00482352) (supplemental Figure 1). To determine whether CD179a was expressed following a month-long course of induction therapy (supplemental Table 1), we obtained 16 paired, day 29 samples for further testing, 7 samples from standard-risk AALL0331, and 9 samples from high-risk AALL0232. All subjects and/or their legally authorized representatives provided written, informed consent in accordance with the Declaration of Helsinki. The study protocol was approved by the COG Cell Bank (AALL18B2-Q), Cancer Therapy Evaluation Program, and Children's Minnesota IRB.

Samples were stained with 2 different antibody combinations (CD20–fluorescein isothiocyanate [FITC]/CD10-phycoerythrin [PE]/CD38-PerCPCy5.5/CD58-APC/CD19-PECy7/CD45-APCH7 and CD9/CD13$^+$33/CD34/CD10/CD19/CD45), including a third tube with SYTO-16 to identify all nucleated cells (COG MRD Panel)[12] that also included a PE-conjugated CD179a mAb (Biolegend, San Diego, CA). In cases where a paired, day 29 sample was available with sufficient viable cells for further sorting, a fourth tube was tested, which included a recombinant FITC-conjugated mAb against CD179a[2] (produced by GenScript, Piscataway, NJ, with FITC-conjugation per the manufacturer's instructions; Abcam, Cambridge, MA). Unlike the FITC-labeled conjugate, the PE-labeled conjugate does not undergo internalization (Biolegend, personal communication).[2] CD3-PerCP, CD10-PE, CD13$^+$/33-APC, and CD19-PECy7 were used as protocol controls (supplemental Table 1); positive and negative controls for the FITC-conjugated CD179a mAb were tested against Nalm6 cells (supplemental Figure 2). All analyses were performed on a Becton-Dickinson FACSCantoII 6-color cell analyzer in a Clinical Laboratory Improvement Amendment/College of American Pathologist–certified laboratory. Cases with ≥20% CD179a surface expression were determined to be positive for χ^2 analyses; all comparisons were performed using GraphPad Prism 8.6 software (San Diego, CA).

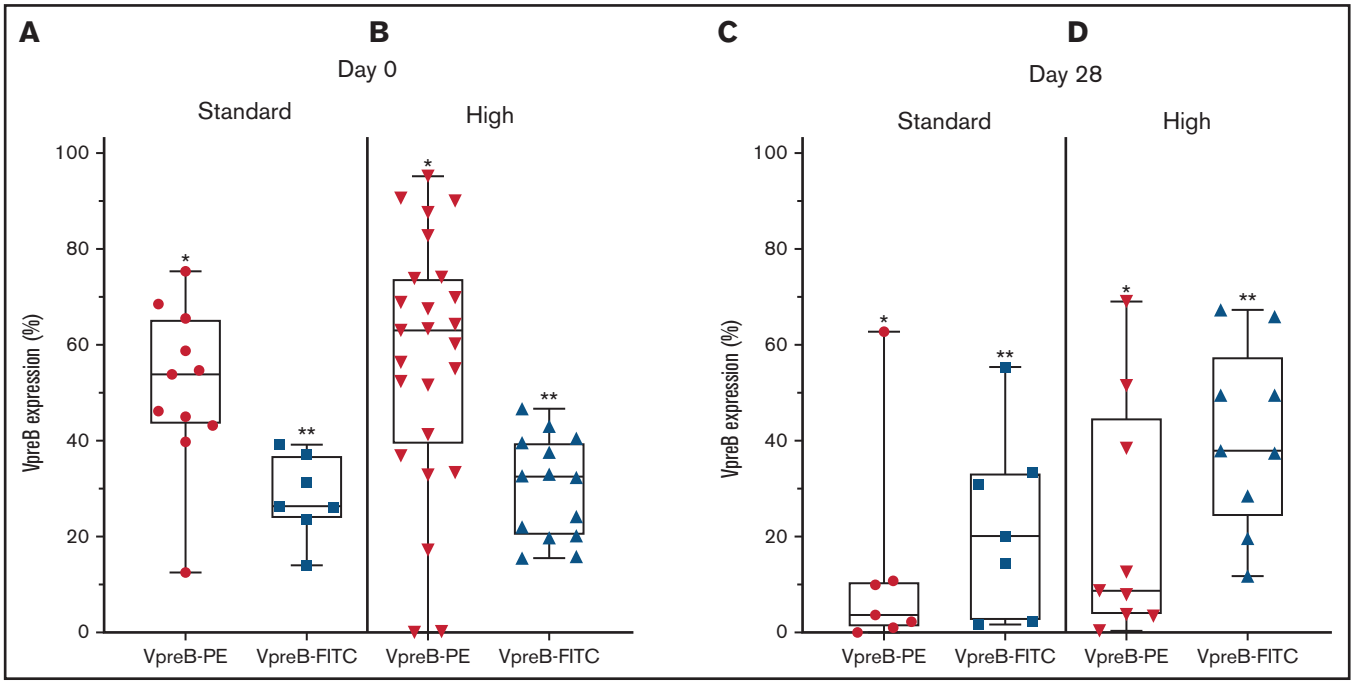

Figure 2. VpreB expression in standard- and high-risk B-ALL at diagnosis (day 0) and end induction (day 29). (A-B) VpreB-PE and VpreB-FITC in standard (A) and high risk (B) showed a spectrum of expression, but brighter expression for the PE conjugate ($P < .001$; unpaired Student t test) at the time of diagnosis. (C-D) VpreB-PE and VpreB-FITC in standard (C) and high risk (D) showed a spectrum of expression but trended to show brighter expression for the FITC conjugate ($P < .001$; unpaired Student t test) at end induction, suggesting that the FITC conjugate might detect recovering marrow populations that include B-lymphoblast populations with hematogones. Statistical comparisons were calculated between combined *PE and **FITC groups.

Results and discussion

We evaluated 36 cases in total: 32 cases were arrested at the $CD10^+$ pre-B stage and 4 cases were arrested at the $CD10^-$ pro-B stage (supplemental Table 2). We found no differences in VpreB expression among 3 developmental stages of B-ALL arrest (Figure 1; P = NS, 1-way analysis of variance). In diagnostic samples, VpreB-PE expression ranged from 0% to 95.2% (55.3% ± 3.9%), and for the FITC-conjugated mAb, expression ranged from 14% to 46.7% (mean, 29.6% ± 2.1%) (Figure 2A-B; unpaired Student t test, $P < .001$). One or both mAbs showed that CD179a was expressed in ≥20% of the B-lymphoblast population for all 36 diagnostic samples, including 3 cases for which RNA-sequencing data were available for comparative analyses (supplemental Table 3).[13] Compared with earlier reports describing ~16% of cases that expressed CD179a in B-ALL,[10] we found that every case expressed CD179a in our series of 36 cases (2-sided Fisher's exact test; $P < .001$). As described by Köhrer et al,[10] we found CD179a expression in cases with *E2A-PBX3* and *KMT2A-R*, but also with *BCR-ABL1*, consistent with Trageser et al,[14] and in other genotypes that may present with ambiguous lineages.[15] Because CD179a was ubiquitously expressed in our series of 36 cases, we hypothesize that it might be broadly targetable in B-ALL, including cases with elevated end-induction MRD.

Because transient dimerization via the SLC is responsible for governing self-autonomous signaling in early B cells, we hypothesized that the abnormal MRD population might escape apoptosis following induction chemotherapy (supplemental Table 4; supplemental Figure 2). Using 2 CD179a-specific mAbs, embedded in the COG MRD panel, we found that both mAbs identified pre-BCR expression in an abnormal B-cell population (Figure 2C-D). For the PE-conjugated mAb, day 29 expression ranged from 0.4% to 69.9% (mean, 18.4% ± 5.9%), and for the FITC-conjugated mAb, expression ranged from 2.0% to 68.1% (mean, 33.4% ± 5.2%; P = .07). PE and FITC photon spectral intensities differ, the former fluorochrome having brighter emission with laser excitation at 488 nm.[16] We speculate that variations in detectable pre-BCR expression between the 2 anti-CD179a mAbs may be attributable to differences between mAb specificity and sensitivity. The CD179a-FITC mAb showed greater detectable expression in the end-induction samples, suggesting that the mAb may have a differential sensitivity/affinity to B-lymphoblasts that co-mingle with an expanding pool of hematogones, which may also be present in a recovering marrow.[17-19]

B-ALLs relapsing after cell-based therapies demonstrate antigen remodeling, downregulation, lineage switches, and T-cell exhaustion.[20] Relapsed or progressive disease in B-ALL may arise from a pervasive genetic/epigenetic reprogramming of B-lymphoblasts in what has been termed "senescence-associated stemness."[9] Because these changes are not reversed with the cessation of induction chemotherapy, relapse-initiating B-lymphoblasts exit senescence-associated stemness and establish therapy-resistant cell populations (identifiable as MRD), which then undergo clonal expansion, leading to relapse and death. These phenomena are especially common in *KMT2A-Rs*, *BCR/ABL1*, and other high-risk molecular lesions.[15] By targeting the autonomous signaling controlled by the pre-BCR, it might be possible to bypass these cell-based mechanisms of relapse.

Because CD19 and CD22 are commonly expressed in B-cell neoplasms, these surface receptors have been logical targets for cell-based therapies (reviewed by Bonifant and Tasian).[5,21] Trials utilizing rituximab have been successful, but have also been difficult to implement into clinical practice, because CD20 is uncommonly expressed in B-ALL.[6] An important limitation for cell-based therapies against CD19, CD20, and CD22 immunotherapies in B-cell neoplasms is their elimination of all normal B cells; this leads to pan B-cell ablation with resultant immune dysregulation. In murine lambda5 and VpreB1/2 knockouts, mature B cells and immunoglobulins reach near-normal levels, suggesting mature B-cell sparing,[22,23] and in humans, SLC expression is absent beyond B cells that express IgM.[24,25] We therefore propose to assess in future clinical trials whether immunotherapies against the pre-BCR might spare a patient's mature B-cell repertoire.

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Authorship

Contribution: S.S.W. and B.S.W. designed the research, analyzed the data, and wrote the paper; C.Q. and C.G.M. provided correlative RNA-sequencing experimental data from a larger study of 633 B-ALL samples; A.M. and N.S. performed the research, which was reviewed and analyzed by G.G.; and N.A.H., A.J.C., and B.L.W. contributed vital expertise to the data analysis and interpretation of COG clinical trial demographic data and its correlation with the experimental data.

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Conflict-of-interest disclosure: The FITC-conjugated anti-VpreB antibody used in this study is protected by US patents 10,858,488 and, for therapeutic allowance, 10,988,533 (S.S.W. and B.S.W.). The remaining authors declare no competing financial interests.

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