Prognostic impact of pretreatment immunoglobulin clonal composition in pediatric B-lymphoblastic leukemia

Refinement of risk-adapted therapy is critical to improving outcomes for childhood B-lymphoblastic leukemia (B-ALL). High-throughput sequencing (HTS) of the immunoglobulin heavy chain (IgH) is a sensitive method for tracking minimal residual disease (MRD).[1-4] It may also offer an opportunity to enhance relapse prediction based on the pretreatment diversity of clonal IgH rearrangements. Using pretreatment IgH HTS data (ClonoSEQ, Adaptive Biotechnologies) from 619 pediatric patients uniformly treated on COG Standard Risk (SR) trial AALL0331 and high-risk (HR) trial AALL0232,[2] we analyzed survival outcomes for patients with B-ALL by their leukemia-associated IgH clonal composition. We found that the number of dominant IgH sequences detectable at diagnosis impacts prognosis particularly among patients with HR B-ALL who lack favorable cytogenetics, suggesting that pretreatment IgH composition may offer an opportunity to refine risk stratification among select groups.

In B-cell development, IgH variable (V), diverse (D), and joining (J) genes recombine to generate unique V-DJ sequences which, when clonally expanded, produce dominant sequences by which leukemia cells can be tracked. Many patients with B-ALL have one to two dominant IgH sequences, but a subset have none and others have three or more.[5] Further, some have numerous subclonal IgH clonotypes as defined by a shared DJ-rearrangement but distinct V genes,[5-7] likely reflecting leukemia development from a common precursor recombining the different V genes subsequent to malignant transformation. In 2018, Wood *et al*. utilized clonal immunoglobulin sequences to report the clinical relevance of end-of-induction (EOI) HTS MRD in pediatric B-ALL.[2] Because post-therapy MRD assessment also requires evaluation of a pretreatment specimen to establish the dominant sequence(s) for subsequent evaluation, this analysis incidentally noted that patients without a dominant, pretreatment IgH sequence appeared to have inferior survival.[2] Thus, IgH composition may bear prognostic relevance independent of its role in MRD monitoring, but this observation has not been explicitly tested in the clinical trial setting.

Using pretreatment IgH HTS data from 603 patients treated on former COG trials (97% of n=619 fulfilling present HTS quality metrics [n=296 from AALL0331: 5.5% of total 5,377 subjects; n=307 from AALL0232: 9.7% of 3,154 subjects]), we analyzed patient characteristics and survival outcomes by IgH composition (Table 1). We found no significant relationship between IgH composition and risk group, age, sex, or CNS status. However, IgH composition did vary within cytogenetic groups. The most prevalent cytogenetic lesions overall were the favorable *ETV6-RUNX1* fusion (13.1%) and double trisomies (DT) of chromosomes 4 and 10 (16.6%). Patients with DT were overall more likely to demonstrate ≥3 IgH sequences (37%) compared to other cytogenetic groups (*ETV6-RUNX1*: 17.7%; other/non-favorable cytogenetics: 19.6%) (P<0.01), potentially due to trisomy of the IgH locus on chromosome 14, as most patients with DT have hyperdiploidy. When separated by trial, however, the association between cytogenetics and number of IgH sequences was only significant among patients on AALL0331 (Table 1).

We found that pretreatment IgH composition is prognostic among select risk groups (Figure 1; Table 1). Patients with no dominant IgH sequences had inferior 5-year event-free survival (EFS) compared to patients with 1-2 (hazard ratio =2.44, 95% confidence interval [CI]: 1.29-4.62) and patients with ≥3 (hazard ratio =4.07, 95% CI: 1.71-9.65). Patients with HR B-ALL appeared solely responsible for this difference, as IgH composition was not associated with prognosis among patients on the SR study AALL0331 (Figure 1A). Among patients on the HR study AALL0232, those with no dominant IgH sequences (n=31) had inferior 5-year EFS (73.4%, 95% CI: 59.2-91.1%) compared to patients with 1-2 (n=215) (5-year EFS 87%, 95% CI: 82.3-91.9, P=0.001), while those with ≥3 (n=61) had superior 5-year EFS (94.8%, 95% CI: 89.2-100, P=0.0003) (Figure 1B). We also found that IgH composition is only predictive of outcome in the absence of favorable cytogenetics (*Online Supplementary Figure S1*).

We next discovered that V-DJ subclone evolution is prevalent but not prognostic. Using the dominant IgH sequence as reference, subclones were defined as sequences with D and J genes identical to the dominant sequence, >2 'N' bases at the D-J junction, and a distinct V gene. Of the 430 patients (71.3%) who had at least 1 dominant sequence fulfilling criteria to assess sequence relatedness, 399 (92.8%) had subclones derived from differential V gene recombination into a common DNJ motif. Cytogenetics differed between patients with subclone evolution and those without (*Online Supplementary Table S1*). Of 31 patients (7.2%) who lacked V-DJ subclone evolution, none had a favorable *ETV6-RUNX1* fusion. Likewise, V-DJ subclone diversity was greater among patients with an *ETV6-RUNX1* fusion compared to patients with DT or other/non-favorable cytogenetics (P=2.5x10^{-5}) (Figure 2A). However, the extent of subclone diversity did not impact 5-year EFS (Figure 2B).

Our findings reveal that, distinct from the use of HTS MRD for relapse prediction, pretreatment IgH HTS data may pro-

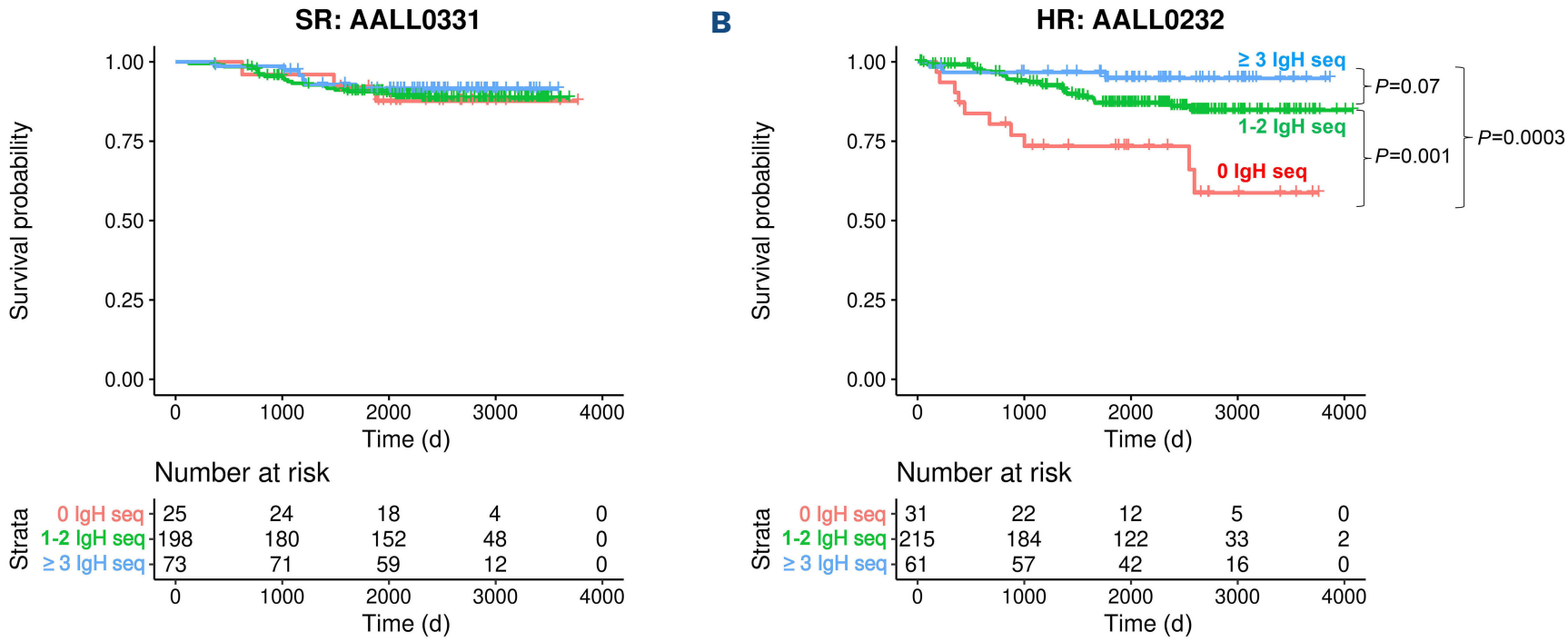

Figure 1. Impact of IgH composition on survival. 5-year event-free survival (EFS) probability is shown according to the number of dominant pretreatment IgH sequences (IgH seq) (0, 1-2, or ≥3) for (A) patients with standard-risk (SR) B-lymphoblastic leukemia (B-ALL) treated on AALL0331 and (B) patients with high-risk (HR) B-ALL treated on AALL0232. IgH composition impacts 5-year EFS in patients with HR B-ALL.

Table 1. Patient characteristics, IgH composition, and survival.

	Standard-Risk AALL0331 N=296				High-Risk AALL0232 N=307			
	0 IgH seq	1-2 IgH seq	≥3 IgH seq	Significance (*P* value)	0 IgH seq	1-2 IgH seq	≥3 IgH seq	Significance (*P* value)
N (%)	25 (44.6)	198 (47.9)	73 (54.5)		31 (55.4)	215 (52.1)	61 (45.5)	
Age in years, mean ± SD	3.6 ± 2.4	3.9 ± 2.3	3.8 ± 2.1	0.60	10.8 ± 5.8	9.6 ± 5.5	8.8 ± 4.9	0.19
WBC x10^9/L, mean ± SD	8.8 ± 8.0	16.4 ± 12.9	13.7 ± 12.2	0.003	77.2 ± 146.4	69.0 ± 85.0	57.2 ± 74.0	0.52
Sex, N (%) Female Male	 16 (64) 9 (36)	 85 (42.9) 113 (57.1)	 31 (42.5) 42 (57.5)	0.12	 13 (41.9) 18 (58.1)	 102 (47.4) 113 (52.6)	 26 (42.6) 35 (57.4)	0.72
CNS status, N (%)* CNS1 CNS2 CNS3	 23 (92) 2 (8) 0	 164 (82.8) 33 (16.7) 1 (0.5)	 55 (75.34) 16 (21.92) 2 (2.74)	0.19	 30 (96.8) 1 (3.2) 0	 178 (82.8) 33 (15.3) 4 (1.9)	 55 (90.2) 6 (9.8) 0	0.11
EOI MRD (FC), N (%)† <0.01% 0.01-<0.10% 0.10-<1.0% ≥1.0%	 16 (64) 6 (24) 1 (4) 2 (8)	 158 (79.8) 19 (9.6) 16 (8.1) 5 (2.5)	 55 (75.34) 9 (12.33) 9 (12.33) 0	0.14	 23 (74.2) 8 (25.8) 0 0	 184 (85.6) 31 (14.4) 0 0	 56 (91.8) 5 (8.2) 0 0	0.07
Cytogenetic group, N (%)†† *ETV6-RUNX1* Double trisomy Unfavorable Neutral Excluded	 0 8 (32.0) 0 14 (56.0) 3 (12.0)	 14 (7.1) 27 (13.6) 2(1.0) 140 (70.7) 15 (7.6)	 4 (5.5) 23 (31.5) 2 (2.7) 41 (56.2) 3 (4.1)	0.02	 5 (16.1) 2 (6.5) 2 (6.5) 17 (54.8) 5 (16.1)	 46 (21.4) 26 (12.1) 6 (2.8) 121 (56.3) 16 (7.4)	 10 (16.4) 14 (33.0) 4 (6.5) 31 (50.8) 2 (3.3)	0.09
5-year EFS, % (95% CI)	92.0 (70.9-98.0)	90.6 (85.3-94.1)	91.5 (81.8-96.2)	0.62	73.4 (51.3-86.7)	87.2 (81.3-91.3)	94.8 (84.2-98.4)	0.0006
5-year OS, % (95% CI)	100 (100-100)	96.8 (92.9-98.6)	97.2 (88.9-99.3)	0.55	90.0 (69.8-96.9)	93.9 (89.2-96.6)	96.7 (86.4-99.2)	0.32

Seq: sequences; WBC: white blood cells; CNS: central nervous system; MRD: minimal residual disease; FC: flow-cytometry; EFS: event-free survival; OS: overall survival; SD: standard deviation; EOI: end of induction; CI: confidence interval. *CNS status definitions: CNS1: absence of blasts in cerebrospinal fluid (CSF); CNS2: <5 WBC/μL CSF and cytospin positive for blasts or >5 WBC/μL but negative by Steinherz/Bleyer algorithm; CNS3: ≥5 WBC/μL CSF and cytospin positive for blasts and/or clinical signs of CNS leukemia. †IgH HTS analysis was limited to patients on AALL0232 with FC MRD <0.10%. ††Patients lacking complete cytogenetic data or with a combination of 2 cytogenetic lesions were excluded. Unfavorable cytogenetics included hypodiploidy, *KMT2A* rearrangements, and *BCR-ABL1* fusions. Note: patient characteristics in this study did not uniformly represent the cytomolecular characteristics of the complete study populations from AALL0331 and AALL0232.

vide independent prognostic information. We observed that the number of dominant IgH sequences detectable at diagnosis was associated with outcome in patients with HR leukemia who lacked favorable cytogenetics, with a particularly inferior outcome among patients without a dominant, pretreatment IgH sequence. Absence of a clonal IgH rearrangement at diagnosis may be biologically relevant. For example, the inferior outcomes of certain subgroups which more often lack a clonal IgH, such as *KMT2A*-rearranged infant leukemia, may relate to leukemic origin from an earlier developmental B-cell stage.[8,9] Our observations support this hypothesis, suggesting that cases undergoing leukemic transformation prior to an initial DJ-recombination event may likewise demonstrate less favorable survival. However, the incidence of *KMT2A* rearrangements in this analysis was too low to test the relationship between this cytogenetic feature and absence of a dominant IgH sequence. Nevertheless, sentinel cytogenetic lesions are prognostic in B-ALL and may correspond to IgH composition. For example, prior reports suggested a lower rate of IgH oligoclonality in patients with an *ETV6-RUNX1* fusion compared to those without a characteristic translocation,[10] highlighting a potential confounding affiliation between favorable cytogenetics and IgH composition. We also observed that *ETV6-RUNX1* leukemias most often show only 1-2 dominant, fully recombined V-DJ sequences. Furthermore, we unexpectedly observed improved survival among patients with HR B-ALL and ≥3 dominant IgH sequences. A greater proportion of patients with DT (37%) had ≥3 IgH sequences compared to other cytogenetic groups. While this finding might raise concern for a confounding effect of favorable cytogenetics on outcome, it was notably only significant in the AALL0331 cohort for whom no survival difference was observed between IgH groups. We also showed that the impact of IgH composition on prognosis was limited to patients who lacked favorable genetics (*Online Supplementary Figure S1*), indicating that cytogenetics alone cannot account for the observed survival impact. Instead, other features – such as B-cell stage at the time of leukemic transformation – may underlie the relationship between IgH composition and prognosis.

The significance of subclone evolution in B-ALL remains to be defined. 'Ordered rearrangement' of the IgH locus involves an initial D-J joining event with V genes available for ongoing rearrangement,[11] resulting in the capacity for subclonal sequences to derive from a common DJ-recombined precursor.[12] For example, Bueno *et al*. did not detect any fully V-DJ-recombined clonal sequence(s) among monozygotic twins with B-ALL.[13] However, there was substantial sequence overlap between the twin cases, suggesting that the leukemia cell of origin arose from a shared DJ-recom-

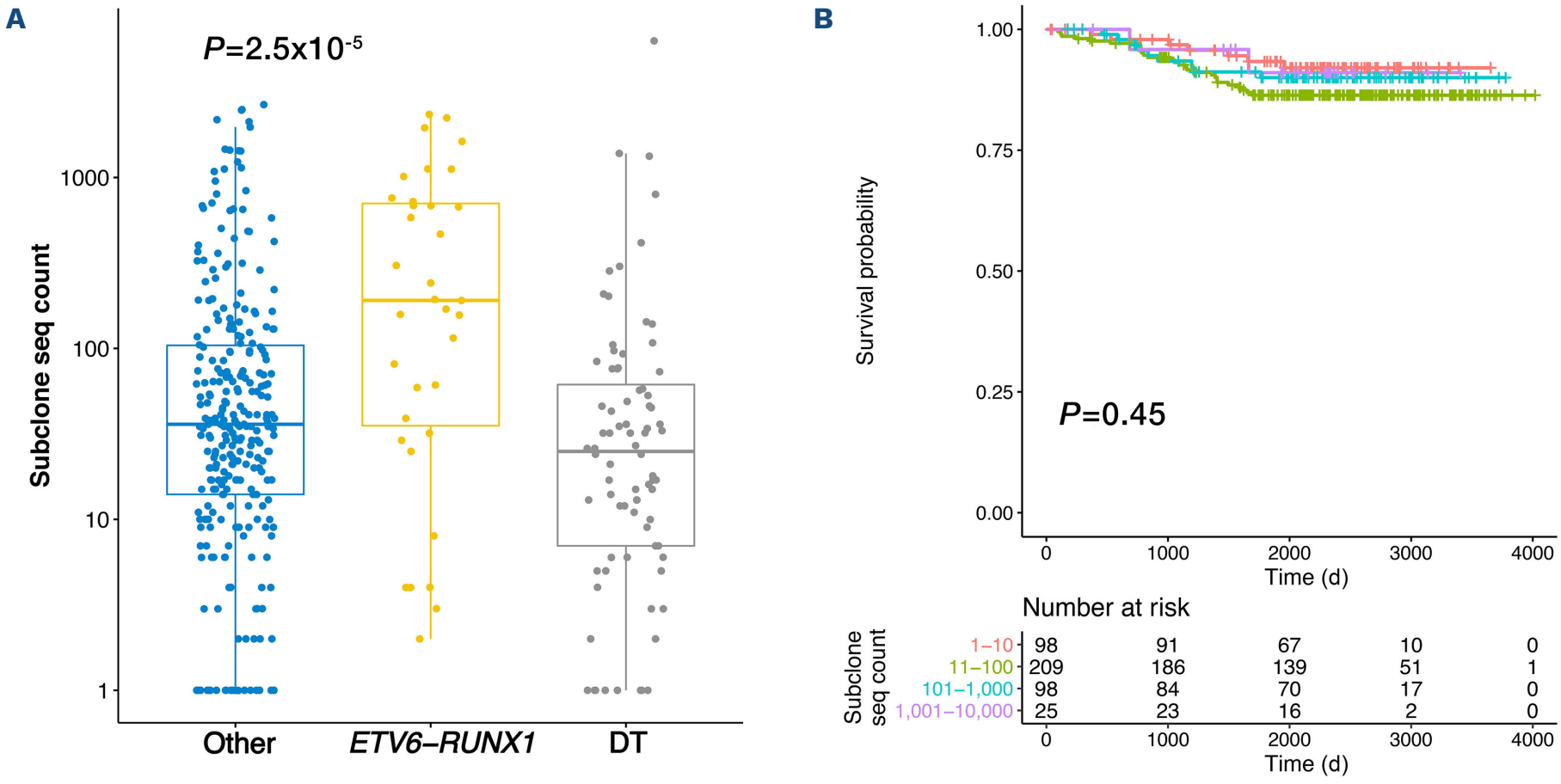

Figure 2. V-DJ subclone diversity. (A) Subclone diversity by cytogenetic group. The total subclone sequence (seq) count (+1; log scale) is shown for 3 cytogenetic cohorts: patients lacking favorable cytogenetics ("Other"), patients with an *ETV6-RUNX1* fusion, and patients with double trisomies (DT) of chromosomes 4 and 10. Patients with B-ALL characterized by an *ETV6-RUNX1* fusion had an overall greater subclone seq count than those with DT or other/non-favorable cytogenetics. (B) Subclone diversity does not impact survival. 5-year event-free survival (EFS) probability is shown according to each patient's total subclone seq count. There was no observed impact of subclone seq count on 5-year EFS. V-DJ sequences: IgH variable (V), diverse (D), and joining (J) genes.

bined progenitor. In 2012, Gawad *et al*. highlighted the vast extent of subclone diversity which can result from this phenomenon, showing a range of 0-4,021 'subclones' per B-ALL.[5] Wu *et al*. likewise observed a range of 0-4,558 evolved subclones,[6] and Bashford-Rogers *et al*. detected ~32 subclones per dominant clonotype, each with entirely distinct V genes.[7] In our analysis, 92.8% of cases (399/430) had subclonal IgH sequences, and 25 (5.8%) had >1,000. Our findings confirm that V-DJ subclone evolution is prevalent in B-ALL and may be greatest in patients with an *ETV6-RUNX1* fusion, but the data do not reveal any prognostic impact of this phenomenon.

This analysis represents the largest uniformly treated population of pediatric patients with B-ALL to date to undergo pretreatment IgH HTS, offering a unique opportunity to test the prognostic impact of IgH composition. Despite our finding that IgH composition impacts outcome in a specific risk group, this retrospective analysis was limited by sample size and selection. Only 56 patients lacked a dominant IgH sequence, which may not have been adequate to account for the confounding impact of other prognostic features in our multivariate analysis. Furthermore, given the design of the study from which the data were obtained,[2] cases were preselected for a HR population with EOI FC MRD ≤0.1%. This enrichment toward a more favorable HR group may have influenced the observed survival impact of IgH composition and precluded our ability to test the relationship to other prognostic features, such as EOI and end-of-consolidation (EOC) MRD. These considerations highlight the importance of future investigations in large, unselected patient cohorts.

In conclusion, pretreatment IgH composition may offer an opportunity to refine risk stratification in B-ALL, particularly in select patients with HR disease, by providing information that enhances the ability of current risk stratification to predict which patients are destined for relapse. Identification of more primitive cell populations may likewise beget novel therapeutic options to improve outcomes.

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Contributions

CF, RER and IRK conceived of the project. LWL and IRK executed the primary data analysis. BW provided correlative data. MD and YD provided biostatistical analysis support. CF interpreted data, drafted the initial version of the manuscript, and edited the manuscript. KRR, SG, MLL, IRK, BW, and RER reviewed and revised the manuscript and provided consultation in data interpretation and analysis.

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Data-sharing statement

The data presented here are available upon reasonable request to the corresponding author subject to COG data-sharing approval.

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