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LETTER

Proximally biased V(D)J recombination in the clonal evolution of IGH alleles in *KMT2A::AFF1* BCP-ALL of all age classes

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We report the analysis of 379 cases of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) using whole-transcriptome sequencing data (Table S1 and Supporting Information S1: Methods). In 48 adult, 36 pediatric, and 37 infant cases of BCP-ALL carrying t(4;11)(q21;q23)/*KMT2A::AFF1* rearrangements (KMT2A-r), we observed strongly favored usage of proximal IGHV genes in V(D)J recombination and more abundant de novo DJ_H recombination than in other BCP-ALL subtypes. This finding has implications for the detection of measurable residual disease (MRD) in clinical practice as MRD-guided treatment choices have proved to provide valuable guidance in a disease that is still associated with poor prognosis in all age categories.

BCP-ALL carrying KMT2A-r is an aggressive leukemia with poor prognosis characterized by the sudden occurrence of very high white blood cell counts, a slight preference for female patients, rapid progression, and significantly shorter survival than KMT2A-r-negative patients.[1,2] Treatment options that significantly prolong the survival of affected patients are limited to allogeneic stem cell transplantation.[2] The incidence of KMT2A-r BCP-ALL is estimated between 4% and 10% in adults and 3% and 4% in childhood.[3]

KMT2A is a histone methyl transferase of the trithorax group involved in the establishment of epigenetic memory. It catalyzes H3K4 methylation of a large number of physiological targets including HOX genes, supporting activation of their expression.[4,5] In leukemogenic fusions, the N-terminal portion of *KMT2A* is fused in frame to more than 100 fusion partners.[6] In the fusion product, the catalytic SET domain of KMT2A is lost. The most frequently observed fusion partners favor the interaction of the fusion product with another histone methyl transferase (DOT1L) that catalyzes H3K79 methylation. As a consequence, proper regulation of target genes activated in this aberrant fashion appears to be disturbed in cells carrying KMT2A-r.[4,5,7,8]

Proximally biased V(D)J recombination was first described in pre-B-cell lines[9] and later shown to depend on *Pax5* function.[10] It is now understood that the generation of a randomized immunoglobulin repertoire depends on chromatin modifications at the IGH locus controlled by *PAX5* and its target, *WAPL*.[11,12] In addition to classical V(D)J recombination,[13] B-cell receptors (BCRs) are known to be modified by direct V_H to V_HDJ_H recombination (V_H replacement)[14,15] and this process is known to occur also in BCP-ALL cells.[16–18] Whether the relative contribution of V(D)J recombination and V_H replacement to clonal evolution of BCRs displays BCP-ALL subtype specificity is currently a matter of investigation.[19,20]

During the analysis of BCR rearrangements in RNA-sequencing (RNA-Seq) data from BCP-ALL samples, we noticed an unusual abundance of rearrangements involving *IGHV6-1* in samples carrying KMT2A-r fusion genes. To quantify this observation, we employed MiXCR analysis of RNA-Seq reads (Figure 1). The number of RNA-Seq reads mapping to a particular IGHV gene by MiXCR was used to quantify the expression levels of individual IGHV genes in a sample. The heatmap in Figure 1 shows the results of this analysis. In this heatmap, the IGHV genes are ordered according to their proximity in terms of genomic distance to the cluster of DJ_H segments in the IGH locus. While for most samples, the expression level of IGHV genes appears to be independent of their genomic position, in KMT2A-r samples the most proximal IGHV gene IGHV6-1 displays significantly higher expression levels as compared to distal ones. This observation is true for KMT2A-r samples of all age classes.

We employed junction analysis of MiXCR-derived nucleotide sequences of rearranged IGH alleles to test whether there are BCP-ALL-subtype-specific differences in V(D)J recombination. IMGT-V-QUEST was used for this purpose, which identifies as junctions the nucleotides coding for the amino acids encompassing

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FIGURE 1 Proximally biased expression of IGHV genes in KMT2A-r B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Heatmap shows samples as rows belonging to the BCP-ALL subtype, indicated by the diagnosis color bar. CLL samples are shown as controls. Each column is an IGHV gene. IGHV genes are sorted according to proximity to the DJ_H cluster of the IGH locus on chr14 with *IGHV6-1* being the most proximal and *IGHV3-76* being the most distal of the IGHV genes shown. IGHV gene expression was measured as the number of RNA-sequencing-derived reads mapping to the IGHV gene as determined by MiXCR, normalized by sample. The expression levels are depicted according to the color scale (−6, 6), with red indicating high expression and blue indicating low expression.

the anchor positions of the CDR3 region (C104-W118) and maps the nucleotides to the V_H-N-D-N-J_H components of the CDR3 region (Table S2). The results are shown in Figure 2. We observed that KMT2A-r samples of all age classes have a median number of three DNJ_H stems per sample (Figure 2A). The only subtype with a similar median number of DNJ_H stems per sample is *t(1;19)/TCF3::PBX1*. At the other end of the spectrum is the *DUX4* subtype where multiple DNJ_H stems per sample were not detected. Furthermore, the CDR3 regions at the nucleotide level identified in KMT2A-r samples are shorter than in most other BCP-ALL subtypes (Figure 2B).

We conclude that V(D)J recombination is proximally biased in samples with KMT2A-r of all age classes. Proximally biased V(D)J recombination leads to preferential usage of *IGHV6-1* in BCR rearrangements. Indeed, up to 50% of samples with KMT2A-r harbor BCRs involving *IGHV6-1*.

We detected subtype specificity in the clonal evolution of BCP-ALL. We observed more DNJ_H stems in KMT2A-r samples as compared to other types of BCP-ALL, and the CDR3 regions in KMT2A-r samples are shorter. This is in line with the observations reported by Darzentas et al.,[19,20] who reported a detailed analysis of clonal evolution in adult BCP-ALL with different contributions of de novo V(D)J recombination and V_H replacement in different BCP-ALL subtypes. V_H replacement can occur repeatedly leading to prolonged CDR3 regions due to footprint nucleotides left behind at each V_H replacement step located between the canonical and the cryptic recombination signal sequence.[20] The present study extends the observation of proximally biased clonal evolution to cases of infant and pediatric BCP-ALL carrying KMT2A-r.

DJ_H recombination is the first step in V(D)J recombination[13] and the realization of a fully randomized immunoglobulin repertoire is dependent on IGH locus decontraction controlled by *PAX5* and *WAPL*.[11,12] Therefore, both the more frequent occurrence of de novo DJ_H recombination as well as the proximal bias in V(D)J recombination suggest a more primitive stage of differentiation of KMT2A-r BCP-ALL. These findings have implications for the clinical management of KMT2A-r BCP-ALL cases as clonal evolution interferes with reliable detection of MRD based on IG rearrangements. An obvious alternative would be the detection of fusion products and co-mutations.

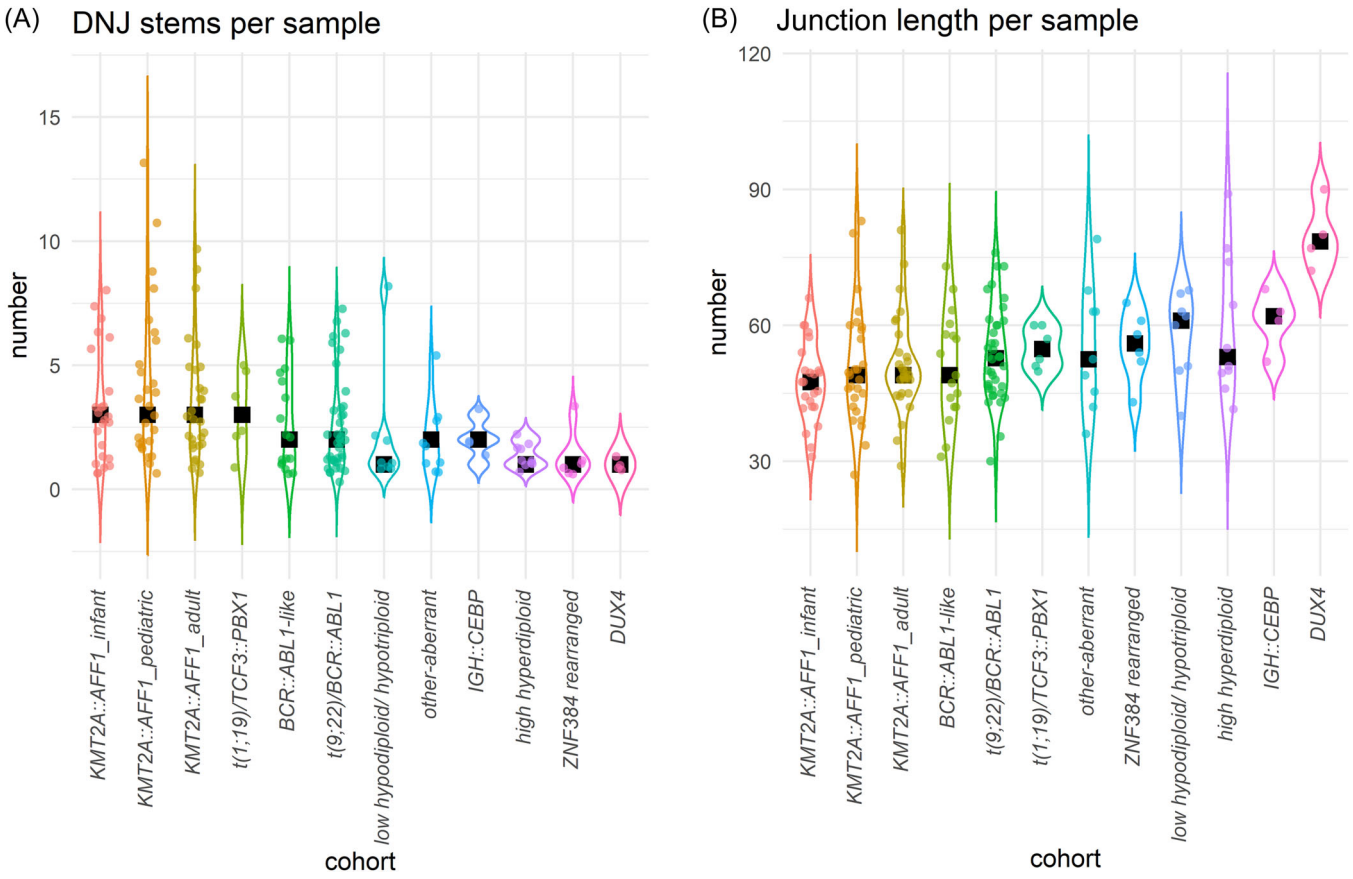

FIGURE 2 Clonal evolution in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) displays subtype specificity. (A) The plot displays the number of different DNJ_H stems per sample belonging to a given BCP-ALL subtype. The black square indicates the median number of different DNJ_H stems. Only BCP-ALL subtypes with a minimum of four samples are shown. (B) The plot displays the distribution of CDR3 lengths per BCP-ALL subtype. The black square indicates the median length of CDR3 regions.

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AUTHOR CONTRIBUTIONS

Heiko Müller performed the analyses and wrote the manuscript. Wencke Walter, Stephan Hutter, Niroshan Nadarajah, and Manja Meggendorfer generated the NGS data. Constance Bär and Frank Dicker contributed expertise in BCR rearrangement analyses. Qingsong Gao, Ilaria Iacobucci, and Charles G. Mullighan provided the WTS data for the validation cohort. Wolfgang Kern, Torsten Haferlach, and Claudia Haferlach supervised and coordinated the research. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

Torsten Haferlach, Claudia Haferlach, and Wolfgang Kern declare part ownership of Munich Leukemia Laboratory (MLL). Heiko Müller, Frank Dicker, Constance Bär, Wencke Walter, Stephan Hutter, Niroshan Nadarajah, and Manja Meggendorfer are employed by the MLL. Charles G. Mullighan received research funding from Loxo Oncology, Pfizer, AbbVie; honoraria from Amgen and Illumina, and holds stock in Amgen.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Britten O, Ragusa D, Tosi S, Kamel YM. MLL-rearranged acute leukemia with t(4;11)(q21;q23)—current treatment options. Is there a role for CAR-T cell therapy? *Cells*. 2019;8(11):1341. doi:10.3390/cells8111341
2. Piciocchi A, Messina M, Elia L, et al. Prognostic impact of KMT2A-AFF1-positivity in 926 BCR-ABL1-negative B-lineage acute lymphoblastic leukemia patients treated in GIMEMA clinical trials since 1996. *Am J Hematol*. 2021;96(9):E334-E338. doi:10.1002/ajh.26253

3. Li J, Dai Y, Wu L, et al. Emerging molecular subtypes and therapeutic targets in B-cell precursor acute lymphoblastic leukemia. *Front Med*. 2021;15(3):347-371. doi:10.1007/s11684-020-0821-6
4. Forgione MO, McClure BJ, Eadie LN, Yeung DT, White DL. KMT2A rearranged acute lymphoblastic leukaemia: unravelling the genomic complexity and heterogeneity of this high-risk disease. *Cancer Lett*. 2020;469:410-418. doi:10.1016/j.canlet.2019.11.005
5. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer*. 2007;7(11):823-833. doi:10.1038/nrc2253
6. Meyer C, Burmeister T, Gröger D, et al. The MLL recombinome of acute leukemias in 2017. *Leukemia*. 2018;32(2):273-284. doi:10.1038/leu.2017.213
7. Chi P, Allis CD, Wang GG. Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer*. 2010;10(7):457-469. doi:10.1038/nrc2876
8. Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R, Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. *Nat Rev Genet*. 2013;14(11):765-780. doi:10.1038/nrg3554
9. Yancopoulos GD, Desiderio SV, Paskind M, Kearney JF, Baltimore D, Alt FW. Preferential utilization of the most JH–proximal VH gene segments in pre-B-cell lines. *Nature*. 1984;311(5988):727-733. doi:10.1038/311727a0
10. Fuxa M, Skok J, Souabni A, Salvagiotto G, Roldan E, Busslinger M. Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes Dev*. 2004;18(4):411-422. doi:10.1101/gad.291504
11. Hill L, Ebert A, Jaritz M, et al. Wapl repression by Pax5 promotes V gene recombination by Igh loop extrusion. *Nature*. 2020;584(7819):142-147. doi:10.1038/s41586-020-2454-y
12. Medvedovic J, Ebert A, Tagoh H, et al. Flexible long-range loops in the VH gene region of the Igh locus facilitate the generation of a diverse antibody repertoire. *Immunity*. 2013;39(2):229-244. doi:10.1016/j.immuni.2013.08.011
13. Jung D, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu Rev Immunol*. 2006;24:541-570. doi:10.1146/annurev.immunol.23.021704.115830
14. Kleinfield R, Hardy RR, Tarlinton D, Dangl J, Herzenberg LA, Weigert M. Recombination between an expressed immunoglobulin heavy-chain gene and a germline variable gene segment in a Ly 1+ B-cell lymphoma. *Nature*. 1986;322(6082):843-846. doi:10.1038/322843a0
15. Reth M, Gehrmann P, Petrac E, Wiese P. A novel VH to VHDJH joining mechanism in heavy-chain-negative (null) pre-B cells results in heavy-chain production. *Nature*. 1986;322(6082):840-842. doi:10.1038/322840a0
16. Choi Y, Greenberg S, Du T, et al. Clonal evolution in B-lineage acute lymphoblastic leukemia by contemporaneous VH–VH gene replacements and VH–DJH gene rearrangements. *Blood*. 1996;87(6):2506-2512. doi:10.1182/blood.V87.6.2506.bloodjournal8762506
17. Steenbergen E, Verhagen O, van Leeuwen E, von dem Borne A, van der Schoot C. Distinct ongoing Ig heavy chain rearrangement processes in childhood B-precursor acute lymphoblastic leukemia. *Blood*. 1993;82(2):581-589. doi:10.1182/blood.V82.2.581.581
18. Wasserman R, Yamada M, Ito Y, et al. VH gene rearrangement events can modify the immunoglobulin heavy chain during progression of B-lineage acute lymphoblastic leukemia. *Blood*. 1992;79(1):223-228. doi:10.1182/blood.V79.1.223.223
19. Darzentas F, Szczepanowski M, Kotrová M, et al. Insights into IGH clonal evolution in BCP-ALL: frequency, mechanisms, associations, and diagnostic implications. *Front Immunol*. 2023;14:1125017. doi:10.3389/fimmu.2023.1125017
20. Darzentas F, Szczepanowski M, Kotrová M, et al. IGH rearrangement evolution in adult KMT2A-rearranged B-cell precursor ALL: implications for cell-of-origin and MRD monitoring. *HemaSphere*. 2023;7(1):e820. doi:10.1097/HS9.0000000000000820