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IGH Rearrangement Evolution in Adult *KMT2A*-rearranged B-cell Precursor ALL: Implications for Cell-of-origin and MRD Monitoring

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B lymphoid neoplasms originate from a single malignantly transformed immune cell, whose immunoglobulin heavy chain (IGH) rearrangement profile is carried by the entire expanded malignant population and mirrors its differentiation status. However, in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and depending on the developmental stage of malignant transformation, multiple new but related IGH rearrangements may result from RAG-mediated recombination that may still be active in the malignant clone. Moreover, such phenomena complicate the identification of molecular minimal residual disease (MRD) markers and have implications for MRD monitoring. Therefore, deep analysis of IGH gene rearrangement patterns and clonal evolution mechanisms—ongoing recombination of incomplete DJ_H rearrangements (V_H-DJ_H) and at a later stage also V_H replacement (V_H-rep), known as receptor editing in physiological B-cell development—may allow new insights into the stage of B-cell differentiation arrest of the leukemia-driving subpopulation that ultimately determines the outcome (Figure 1A), and provide more accurate MRD results.[1–5]

KMT2A-rearranged (*KMT2A*^{rearranged}) BCP-ALL is a common occurrence in infant B-ALL affecting more than 70% of cases. It is associated with an aggressive disease course, high-risk status, and poor prognosis. Recent studies have reported immature features of the putative cell-of-origin in *KMT2A*^{rearranged} cases.[6–8] Single-cell transcriptome comparison between lymphoblasts and hematopoietic stem cells inferred that most *KMT2A*^{rearranged} cases feature an early lymphocyte precursor stage.[7] In another study, analysis of paired diagnostic and relapse samples revealed that relapses often emerge from minor subclones that were already present at diagnosis,[6] where such highly subclonal architecture points to maturation arrest at a very early state with persistent IGH recombination.

Much less is known about the putative cell-of-origin in adult *KMT2A*^{rearranged} BCP-ALL. We hypothesized that *KMT2A*^{rearranged} cases—correlating with high-risk clinical features and corresponding to early-stage pro-B ALL—would show stem cell proximity not only in infants but also in adults. For this purpose, we studied IGH gene rearrangement profiles in 18 *KMT2A*^{rearranged} cases and compared them to those of 137 cases of other molecular BCP-ALL subtypes without *KMT2A* gene fusions (summarized as *KMT2A*^{germline} and depicted in Suppl. Figure S1).

The information on the presence of a *KMT2A* fusion and corresponding subtype allocation was extracted from transcriptome sequencing performed according to published protocols.[9] Patients were treated according to protocols of the German Multicenter Study Group on Adult Acute Lymphoblastic Leukemia (GMALL). For details on the patient cohort, see Suppl. Table S1. Amplicon-based NGS with EuroClonality-NGS IGH-VJ-FR1 and IGH-DJ primers and the EuroClonality-NGS central in-tube quality/quantification control was performed using 2-step PCR and 500 ng DNA.[10] Samples were sequenced on a MiSeq (Illumina, San Diego, CA) with 2 × 250 bp reads. Sequences were analyzed with ARResT/Interrogate.[11] For IGH clonal evolution assessment, we isolated the "DNJ-stem" (or simply "stem") of nucleotide junctions (Figure 1B and Suppl. Materials and Methods). Thus, clonotypes sharing the same stem were considered clonally related and underwent further analysis, including a determination of whether the stem should be considered stable or evolving (see Suppl. Materials and Methods). The stem was considered "malignant" if found in ≥5% of sample reads and/or evolving at any abundance.

We detected exceptionally higher rates of clonal evolution in *KMT2A*^{rearranged} (17/18, 94%) than in *KMT2A*^{germline} cases (37/137, 27%). Additionally, we observed that the V_H-DJ_H and V_H-rep mechanisms were unevenly distributed between 68 evolving stems in 18 *KMT2A*^{rearranged} cases and 63 evolving stems in 137 *KMT2A*^{germline} cases: while 16 of 18 (89%) *KMT2A*^{rearranged} patients showed ongoing V_H-DJ_H and virtually no V_H-rep-driven clonal evolution (2/68, 3% of evolving stems), V_H-rep was a common phenomenon in the *KMT2A*^{germline} group (21/63,

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Supplemental digital content is available for this article.

HemaSphere (2023) 7:1(e820).
http://dx.doi.org/10.1097/HS9.0000000000000820.
Received: September 2, 2022 / Accepted: November 18, 2022

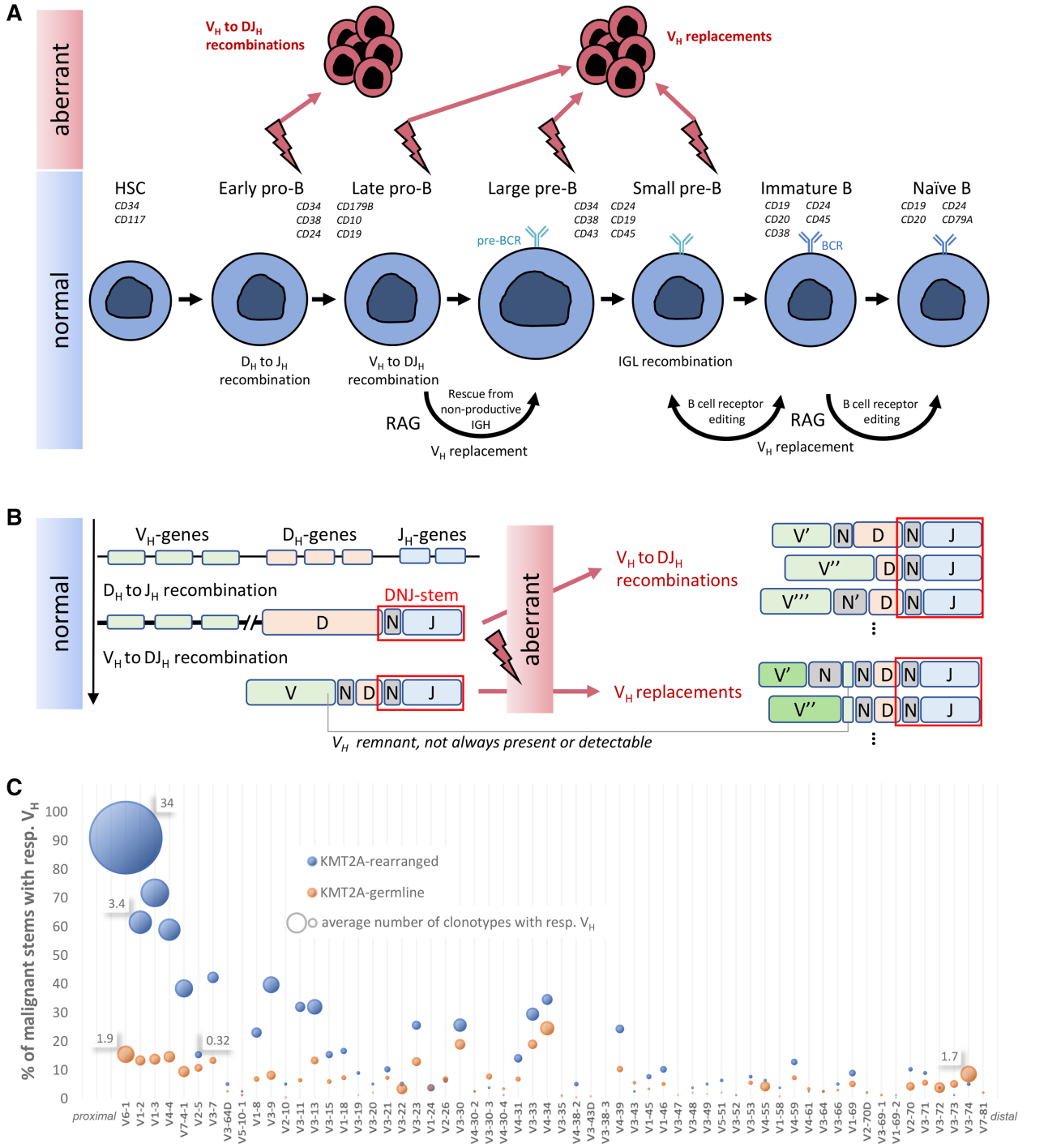

Figure 1. Overview of the research hypothesis and results. (A) Normal B-cell development and a proposed schema for aberrant activity in the pathogenesis of BCP-ALL. (B) IGH gene recombination and V_H replacement schematic. Incomplete and complete IGH rearrangements may provide the templates for aberrant multiple instances of V_H to DJ_H recombinations and V_H replacements. The DNJ-stem is highlighted in red rectangle on the rearrangement junctions, and is the same in all instances. Different genes or N regions across junctions are indicated with prime symbols. (C) V_H usage of malignant stems in *KMT2A*^{rearranged} and *KMT2A*^{germline} cases. x-axis: V_H genes according to their order on IGH locus starting from J_H-proximal; y-axis: percentage of malignant stems with clonotypes featuring respective V_H (sum for each category exceeds 100% because evolving stems have multiple clonotypes); bubble area size: average number of such clonotypes in such stems, with select values provided for size reference. BCP-ALL = B-cell precursor acute lymphoblastic leukemia; IGH = immunoglobulin heavy chain.

33% of evolving stems) (Table 1, top part). Strikingly, the only *KMT2A*^{rearranged} case without any kind of clonal evolution harbored an atypical hitherto not described *KMT2A::UBASH3B* in-frame gene fusion, which spans 4.3 Mbp on chromosome 11q, suggesting an interstitial deletion as a mechanism of origin (Suppl. Figure S2). A *KMT2A* gene fusion originating from interstitial deletion (*KMT2A::ARHGEF12*) has been described in rare cases of therapy-related AML and ALL as well as high-grade B-cell lymphoma identifying *KMT2A* deletions as driver mechanism in very specific circumstances.[12] The other 17 *KMT2A*^{rearranged} cases showed typical *KMT2A* fusions (*KMT2A::AFF1* [16/18, 89%] or *KMT2A::MLLT1* [1/18, 6%]) (Suppl. Table S1A).

Table 1

IGH clonotype and clonal evolution characteristics in the *KMT2A*^{rearranged} and *KMT2A*^{germline} BCP-ALL groups

Data rows in bold indicate statistically significant (p-value <0.05) differences in the nonparametric Mann-Whitney statistical test. * The only KMT2Arearranged case without signs of clonal evolution harboured an atypical hitherto not described KMT2A::UBASH3B fusion. ** Unambiguous assignment to clonal evolution mechanism is not possible in two cases.

	KMT2A^{rearranged}		*KMT2A*^{germline}		
	percentage	*count*	*percentage*	*count*	*p-value*
cases with malignant clonotype(s)	100%	18/18	92%	126/137	0.365
...in IGH-VJ library	100%	18/18	88%	121/137	0.219
...in IGH-DJ library	**89%**	**16/18**	**47%**	**65/137**	**0.001**
cases with any type of clonal evolution	**94%**	***17/18**	**27%**	****37/137**	**<0.001**
...cases with V_H-DJ_H	**89%**	**16/18**	**12%**	**17/137**	**<0.001**
...cases with V_H-rep	11%	2/18	13%	18/137	>0.999
...stems with V_H-DJ_H	**97%**	**66/68**	**63%**	****40/63**	**<0.001**
...stems with V_H-rep	**3%**	**2/68**	**33%**	**21/63**	**<0.001**
across each group, all stems	*median*	*min-max*	*median*	*min-max*	*p-value*
number of related clonotypes per stem	**118**	**1-1288**	**5**	**1-499**	**<0.001**
abundance of most popular related clonotype per stem, %reads	**0.5**	**0.03-91**	**40**	**0.02-99**	**<0.001**

BCP-ALL = B-cell precursor acute lymphoblastic leukemia; IGH = immunoglobulin heavy chain.

Underlying that the vast majority of *KMT2A*^{rearranged} evolving stems displayed ongoing V_H to DJ_H recombination, 41 of 68 (60%) *KMT2A*^{rearranged} compared to 24 of 63 (38%) *KMT2A*^{germline} evolving stems were detected in the IGH-DJ library as well, providing additional support for the hypothesis that the origin and malignant driver of *KMT2A*^{rearranged} BCP-ALL is a progenitor-like population with an incomplete DJ_H rearrangement. This has important implications for IG-rearrangement-based MRD monitoring, especially in *KMT2A*^{rearranged} cases, in which both IGH-VJ and IGH-DJ tubes should be sequenced for follow-up samples and checked for the presence of both the initially dominant and any clonally related evolved clonotypes that together compose the entirety of MRD.

We further noticed a remarkable difference in the V_H gene usage between the groups. *KMT2A*^{rearranged} cases showed a preferential and biased usage of the most J_H-proximal human V_H gene, V_H6-1: 91% of malignant stems in the *KMT2A*^{rearranged} group harbored at least one V_H6-1-rearranged clonotype—in the *KMT2A*^{germline} group, this was the case in only 16%. Beyond that, *KMT2A*^{rearranged} malignant stems with a V_H6-1 gene had exceptionally high numbers of clonally related clonotypes, both compared to *KMT2A*^{germline} stems with a V_H6-1 gene but also compared to stems with further downstream V_H genes (Figure 1C). Generally, *KMT2A*^{rearranged} evolving stems showed higher numbers and lower median abundance of related clonotypes, suggesting that V_H-DJ_H is a highly active and ongoing process in *KMT2A*^{rearranged} BCP-ALL (Table 1, bottom part).

Overall, 16 of 18 *KMT2A*^{rearranged} ALLs had the immunophenotype of a pro-B ALL, all with evolving stems, whereas in the *KMT2A*^{germline} group, only 11 of 137 cases corresponded to a pro-B-ALL, of which only one case had evolving stems (Suppl. Table S1). These data suggest that the stem cell proximity of the IGH genotype of *KMT2A*^{rearranged} ALL is not fully explained by the immature immunophenotype.

We conclude that the 2 mechanisms driving IGH clonal evolution in BCP-ALL, ongoing V_H-DJ_H recombination and V_H replacement, correlate with maturation arrest in different stages of B-cell development. The latter is thought to be a physiologically legitimate rescue mechanism for pre-B cells with nonfunctional or autoreactive IGH rearrangements and has recently been linked to B-cell receptor-mediated signaling in human immature B cells.[13] Ongoing V_H-DJ_H recombination, on the other hand, is a sign of a differentiation arrest in a very early stage and takes place in virtually all BCP-ALL cases with typical *KMT2A* fusions, consistent with findings stating that leukemic blasts in *KMT2A*^{rearranged} ALL originate from early precursor cells.[8] Consentaneous with this finding, V_H6-1, ubiquitously used in our V_H-DJ_H-evolving *KMT2A*^{rearranged} group, was found in fetal liver at high clonotypic abundances as part of a very early immune system ontogeny; those V_H6-1-rearranged B cells reportedly persist into adulthood in the course of life-long, innate B lymphopoiesis and are thought to serve as founders of malignant transformation late in life.[14] As yet another sign of fetal origin of that group, the preferential usage of D_H7-27 was described in fetal human liver early B cells[15]—in our analysis, 9% of stems used D_H7-27 in the *KMT2A*^{rearranged} group compared to only 1% in the *KMT2A*^{germline} group.

In summary, we provide evidence that the cell-of-origin and malignant driver of *KMT2A*^{rearranged} BCP-ALL, an aggressive disease with dismal outcome and increased risk of relapse,[8] show ontogenic stem cell proximity not only in infants but also in adults. Furthermore, we report on striking similarities of its IGH rearrangement profile to fetal B cells, linking *KMT2A*^{rearranged} BCP-ALL to fetal-derived early immune system ontogeny.

We also share a note of caution. The high number and low abundance of clonally related clonotypes in evolving cases have the potential to complicate or even impede both marker identification and MRD monitoring: all individual clonotypes of evolving stems may fall below traditional screening abundance thresholds at diagnosis, and different individual but related clonotypes may persist in follow-up timepoints. We therefore recommend to always analyze both IGH-VJ and IGH-DJ tubes in BCP-ALL and to check for the presence of clonally related clonotypes to avoid false-negative or underestimated MRD values, especially in *KMT2A*^{rearranged} cases with a high propensity for clonal evolution of IGH rearrangements as presented herein. Accordingly, when available, the *KMT2A* chromosomal breakpoint should be considered as a more stable and specific MRD marker.

ACKNOWLEDGMENTS

The authors thank the Hematology Laboratory Kiel staff for sample processing. The authors are indebted to the GMALL Trial Center (R. Reutzel, C. Fuchs) and participating hospitals for patient recruitment, care and logistics.

AUTHOR CONTRIBUTIONS

ND and MB shared senior authorship. MB and MS designed the research; NG supervised the clinical trial; MK, ND, and FD processed, analyzed, and interpreted high-throughput sequencing data; ND and KP worked on ARResT/Interrogate; LB, AH, TB, and MN analyzed and interpreted RNA-Seq data; FD, ND, MKe, and MS performed statistical analyses; MB, MS, and CDB supervised the project; FD, ND, MS, and MB drafted the first version of the manuscript; all authors read and accepted the final version of the manuscript.

DISCLOSURES

MB received personal fees from Incyte (advisory board) and Roche Pharma AG, financial support for reference diagnostics from Affimed and Regeneron, grants and personal fees from Amgen (advisory board, speakers bureau, travel support), and personal fees from Janssen (speakers bureau), all outside the submitted work. MS received a personal fee from Amgen (speakers bureau), outside the submitted work. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. All the other authors have no conflicts of interest to disclose.

SOURCES OF FUNDING

This work was supported by the Deutsche José Carreras Leukämie-Stiftung (grants DJCLS R 15/11 to MB and DJCLS 06R/2019 to MS and MB, as well as by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) project number 444949889 (KFO 5010/1 Clinical Research Unit "CATCH ALL") to LB, AH, MN, MB, and CDB.

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