Review

Genomics and precision medicine in pediatric acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in the pediatric population, accounting for about 25% of childhood cancers. Drastic therapeutic improvements have been made for pediatric ALL since the early 1960s, marking the most successful treatment paradigm in pediatric oncology. The clinical success derived from refined risk-adapted therapy based on presenting features, cytogenetics and minimal residual disease, prevention of central nervous system relapse, and improvement of supportive care measures. With contemporary therapies, survival of children with ALL now exceeds 90%. However, ALL represents one of

leading causes of cancer-related death, as 15-20% of patients continue to relapse and outcomes post-relapse remain poor. Since the early 2000s, large-scale genomic studies of ALL, greatly facilitated by the advent of next generation sequencing (NGS), have enabled the development of a novel taxonomy for ALL in the molecular era. The access to NGS technologies identifies novel ALL subsets characterized by "driver" oncogenic alterations, previously cryptic on conventional karyotyping methods. With genomic characterization, the group of formerly unclassified B-lineage ALL reduces from 25% to a marginal 5% of ALL. The revised molecular classification of ALL confers prognostic significance and describes the predilection of unfavorable ALL subtypes with increasing age, partially elucidating the worst outcome of adolescents and young adults with ALL. Large-scale genomic analysis also reveals inherited alterations predisposing to ALL occurrence or to different drugs' sensitivities. Most importantly, the genomic portrait of ALL uncovers novel therapeutic vulnerabilities, paving the way towards precision medicine opportunities in ALL.

Key words: Acute lymphoblastic leukemia, childhood leukemia, genomics, precision medicine, targeted therapies

INTRODUCTION

In the last 60 years, substantial progress has been made in the management of pediatric acute lymphoblastic leukemia (ALL) that translated into meaningful survival improvement. With modern therapies, more than 90% of children with ALL now become long-term survivors [1]. The key factor for this success was the refinement of multi-agent chemotherapy regimens' delivery via enrollment of thousands of children with ALL onto prospective randomized clinical trials. Results of these clinical trials define the current standard of care and highlight the importance of central nervous system (CNS)-directed therapy and risk-adapted therapy based on patient's presenting characteristics, leukemia biology and early response as measured by minimal residual disease (MRD) [2, 3]. Clinical characteristics encompassing from age and leukocyte count at diagnosis, leukemia immunophenotype to extramedullary disease involvement, have been first pinpointed as prognostic factors and universally integrated for risk stratification and

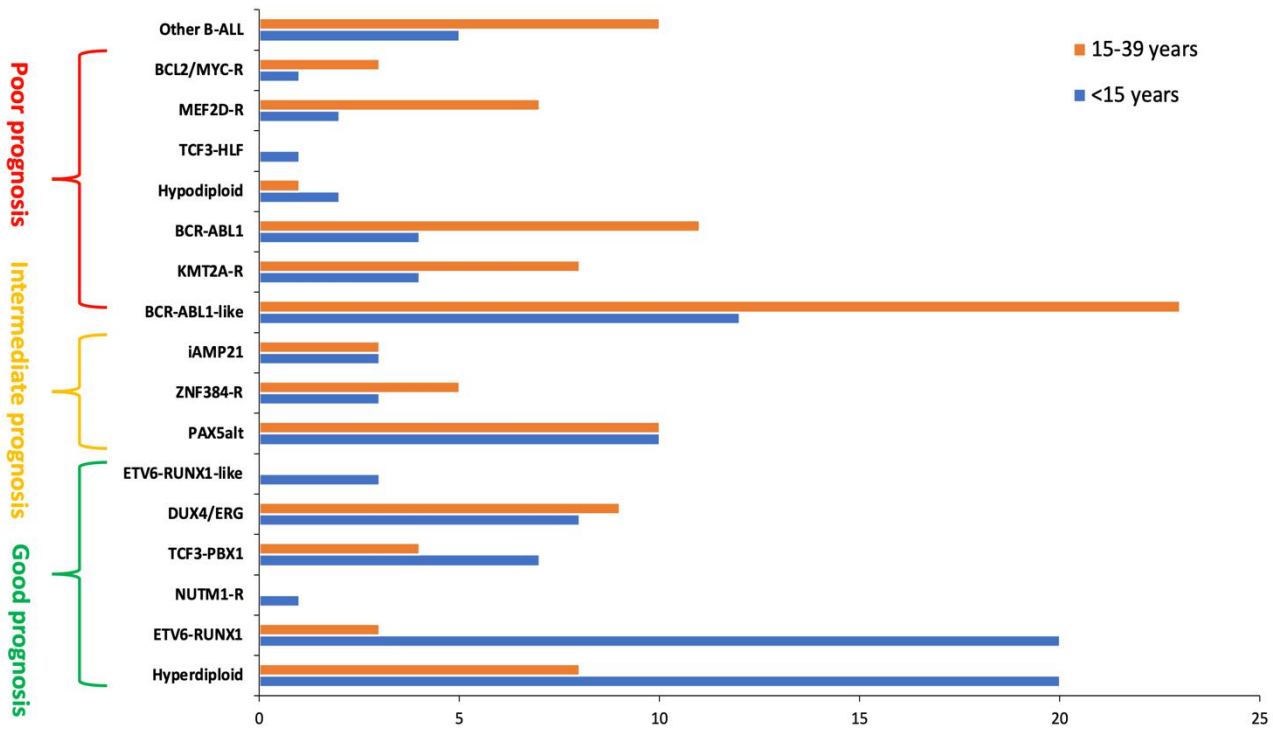
treatment assignment for childhood ALL. For instance, age ≥ 10 years at diagnosis and presenting leukocyte count ≥ $50x10^9$/L (the National Cancer Institute (NCI)-Rome criteria), as well as extramedullary disease and T-cell phenotype classify the patients in the high-risk (HR) group due to lower survival [4]. Sentinel genetic alterations, characterized by chromosomal aneuploidies or rearrangements via conventional cytogenetics, represent an essential component of risk stratification. Some alterations are associated with favorable prognosis, such as high hyperdiploidy (51-65 chromosomes) and *ETV6-RUNX1*; while other cytogenetic abnormalities including low hypodiploidy (<40 chromosomes) or near-haploidy, intrachromosomal amplification of chromosome 21 (iAMP21), *BCR-ABL1* or Philadelphia chromosome (Ph^+), *KMT2A* (formerly known as *MLL*) rearrangement (KMT2Ar) and *TCF3-HLF* confer a worse prognosis [5-7]. Risk-group stratification based on the patient's clinical and molecular characteristics for tailored therapy intensification has contributed to the dramatic improvement of pediatric ALL's prognosis over the last 50 years [8]. However, up to 25% of B-lineage ALL (B-ALL) remain unclassified by conventional cytogenetics and is referred to as B-Others [9]. The advent of high-throughput genomic approaches has marked a new paradigm in ALL characterization, revealing a diverse spectrum of subtype-defining alterations that were missed due to their cryptic nature or undetectable by orthogonal methods. These novel alterations can be divided into three different categories : 1) sequence mutations affecting transcription factors (e.g. *PAX5* P80R, *IKZF1* N159Y); 2) recurrent rearrangement of a single gene with multiple partners (e.g. *ZNF384*, *MEF2D* and *NUTM1*-rearranged ALL); 3) a range of different alterations involving multiples genes within the same molecular group (e.g. *PAX5* alterations, *DUX4/ERG* subtype, *ETV6-RUNX1*-like ALL, or Philadelphia chromosome-like (Ph-like) ALL) [8-11]. Next-generation sequencing (NGS) platforms and large-scale genome-wide studies, especially microarray for copy number alterations (CNAs) and whole-transcriptome analysis, display a high ability to classify new molecular subgroups based on their gene expression profiles (GEP). This approach has unraveled new oncogenic drivers of leukemogenesis. Many of them have been shown to have prognostic and/or therapeutic implications [2, 9]. The frequency of each molecular subgroup varies with age; thus partially elucidates the age-related differential outcome in ALL [10]. In this review, we aim to provide an overview of the most recent advances in ALL genomics, and to highlight the prognostic impact and therapeutic opportunities derived from this modern classification. It must be specified that some of these novel entities discussed herein

should be considered as provisional. Their prognostic and therapeutic significance will require further validation in large, prospective and uniformly-treated patient cohorts.

MOLECULAR CLASSIFICATION OF ALL

B-ALL

In the early 2000s, Yeoh *et al.* confirmed that GEP is able to accurately classify known cytogenetic subgroups and sometimes, rectify karyotyping misclassification for the *ETV6-RUNX1* subgroup. They also highlighted unique gene expression phenotypes identifying novel subgroups for previously unclassified B-ALL [12]. Furthermore, gene expression clustering correlates with outcome and can be used for prognostic and risk-group classification [13, 14]. Herein, we will only emphasize on the recently discovered molecular subgroups, their potential prognostic association and their predilection according to age group. **Figure 1** summarizes the B-ALL subgroups with their distribution by age group and a preliminary proposition of risk-group classification.



significance of rare and novel ALL subtypes can be influenced by treatment strategies and will require further confirmation in larger studies.

Ph-like ALL

The discovery of Ph-like or BCR-ABL1-like ALL hails from genomic exploration of HR B-ALL [13-17]. This subgroup is defined by an activated kinase gene expression profile, similar to that of Ph^+ ALL but missing the canonical *BCR-ABL1* fusion. This subset was identified in 2009 by two independent ALL cohorts defined by two distinct gene classifiers that shared only 7 genes in common [15, 16]. Interestingly, Ph-like ALL is associated with a worse prognosis than other HR B-ALL and comparable to that of Ph^+ ALL [15]. In multivariable analysis, the prognostic significance of Ph-like ALL was retained as an independent adverse outcome biomarker for relapse [18]. This subgroup accounts for ~15% of pediatric B-ALL and increases with age and risk group. In younger children, it represents 10% of standard-risk (SR) ALL and 13% among HR-ALL. The prevalence increases to 21% among adolescents and 27% in young adults and then stabilizes around 20% in older adults after the age of 40 [19, 20]. Alterations (deletions and inactivating mutations) of *IKZF1* or other lymphoid transcription factors (*CDKN2A/B, PAX5, ERG*, *ETV6*) and kinase-activating alterations constitute the molecular hallmark of Ph-like ALL [13, 14, 16, 19]. *CRLF2* rearrangements (e.g. *IGH-CRLF2* and *P2RY8-CRLF2*), conferring CRLF2 overexpression, comprise half of Ph-like ALL, often harbor concomitant *JAK* mutations or other JAK-STAT pathway alterations (*SH2B3*, *IL7R*) in about 50% of the *CRLF2*-rearranged cases [19]. *CRLF2* rearrangements are associated with a worse prognosis and are more frequent in older children and in people with Hispanic or Native American origin [17]. Overall, more than 90% of Ph-like ALL harbor a myriad of kinase-activating alterations that can be further divided in 2 major categories: 1) alterations activating JAK-STAT signaling pathways, predominantly rearrangements of *CRLF2*, *JAK2* and *EPOR*; 2) translocations involving ABL-class genes (*ABL1, ABL2, CSF1R, PDGFRA, PDGFRB, LYN*). A small number of Ras pathway (*KRAS, NRAS, PTPN11, NF1*) mutations have been identified; nevertheless, it remains unclear whether these mutations represent pathogenic drivers in Ph-like ALL or only contribute to a similar kinase-activated signature. In addition, some rare fusions involving other tyrosine kinases such as *NTRK3, DKGH* or *FLT3* have been reported in Ph-like ALL [10, 19, 20]. *IKZF1* (gene encoding for the transcription factor IKAROS) intragenic deletions and inactivating mutations are

preponderant in kinase-activating leukemia such as Ph^+ or Ph-like ALL [14, 16, 21, 22]. *IKZF1* alterations are present in up to 70% of Ph^+ ALL and Ph-like ALL and confer a worse prognosis compared to their respective counterparts with wild-type *IKZF1* [19, 23]. Nevertheless, the independent negative prognostic impact of *IKZF1* deletion in B-ALL is still debatable. *IKZF1* was first described as a pejorative marker for the occurrence of relapse [16], but multivariable analyses across different consortia had come to conflicting conclusions. In the Dana Farber Cancer Institute (DFCI) ALL 05-001 study, *IKZF1* deletion was associated with poor survival irrespective of the presence of kinase fusion and MRD among 105 NCI HR B-ALL cases [7, 23]. However, the results from Children's Oncology Group (COG) P9905/P9906 trials failed to confirm the prognostic impact of *IKZF1* alterations in multivariable analysis when analyzed by risk group [22, 24]. The enrichment of *IKZF1* alterations in the already known unfavorable Ph-like ALL represent major confounders and contribute to the uncertain prognostic impact of *IKZF1*. A new category, called $IKZF1^{plus}$, regrouping *IKZF1* deletions that co-occur with *CDKN2A*, *CDKN2B*, *PAX5* or *PAR1* deletion in the absence of *ERG* deletion could represent a more homogeneous and discriminative subset with high risk of relapse [25].

DUX4-rearranged and ERG-deregulated ALL

Gene expression profiling of HR B-ALL from the COG P9906 cohort discovered a new cluster with an excellent prognosis, contrasting with the overall outcome of HR ALL. This subgroup presents with recurrent intragenic *ERG* deletion [14], and has been later identified as *DUX4*-rearranged and *ERG*-dysregulated (*DUX4*/*ERG*) subtype, accounting for 4 to 7% of B-ALL [26, 27]. All cases within this subgroup harbor a rearrangement of *DUX4* to the immunoglobulin heavy chain (*IGH*) gene or to *ERG*, leading to *DUX4* activation and overexpression. *DUX4* encodes a double homeobox transcription factor that is not expressed in normal human B-cell development. Its activation is responsible for ERG deregulation and loss of function. Beside frequent intragenic *ERG* gene deletion (about 50% of the cases), recurrent alterations in other lymphoid transcription factors are present, including *IKZF1* alterations in one third of cases. *DUX4*/*ERG* subtype is encountered more often in childhood HR ALL (9.4% vs. 5.2% in SR group) and in adolescents (10.2%); however, its prognosis appears to be excellent, despite the co-existence of *IKZF1* deletion [26-29].

ETV6-RUNX1-like ALL

ETV6-RUNX1-like ALL subset regroups B-ALL cases sharing a similar GEP with *ETV6-RUNX1*-positive ALL but lacking the *ETV6-RUNX1* gene fusion. This subgroup represents about 3% of B-ALL and seems to be almost exclusive to childhood ALL [11, 27]. They are characterized by frequent co-existing aberrations (gene fusion or copy number alteration) of *ETV6* and *IKZF1* genes [27, 30]. Similar to *ETV6-RUNX1* ALL, the *ETV6-RUNX1*-like phenotype is also associated with a favorable prognosis [27, 31].

PAX5-driven subtypes

Aberrations involving the transcription factor *PAX5* has recently been individualized as a founding event in B-lymphoid leukemogenesis [11, 31]. Two distinct subgroups have been identified within *PAX5*-driven mutations: *PAX5* altered (*PAX5*alt) ALL, that comprises a diverse spectrum of *PAX5* rearrangement, intragenic amplification or sequence mutation, and *PAX5* P80R. The latter is characterized by the deleterious *PAX5* P80R point mutation coexisting with a near systematic inactivation of the wild-type *PAX5* allele either by deletion, loss-of-function mutation or loss of heterozygosity. Frequent signaling pathway mutations arise concomitantly with the *PAX5* P80R subtype, mostly involving RAS and JAK-STAT pathways [11]. The *PAX5*alt subtype predominates in childhood ALL (10%) compared to adult ALL (7%) and is associated with intermediate prognosis [31]. In contrast, *PAX5* P80R increases in frequency with age and also confers an intermediate prognosis [11].

MEF2D and ZNF384 rearrangements

The identification of recurrent fusions involving *MEF2D* and *ZNF384* highly suggests their role as oncogenic drivers in B-ALL. Both *MEF2D* and *ZNF384*-rearranged (*MEF2D*r and *ZNF384*r) ALL harbor a profile of activated transcription factor gene and disruption in B-cell development, but still present a distinct GEP allowing for the definition of two new subgroups [31, 32]. *MEFD2*r and *ZNF384*r resemble by their multiple fusion partners, the most common being *BCL9* for the former and *EP300* for the latter [33, 34]. These rare subsets are found in adolescents and young adults (AYA) more often than in younger children, each subset totaling roughly 7% of B-ALL in AYA and 4% in children [32, 34]. The scarcity of these subgroups lessens the ability to accurately determine their prognostic significance; however, *MEFD2* fusions appear to confer a

poor prognosis, while *ZNF384* fusions are associated with an intermediate prognosis [31, 32]. Interestingly, their molecular signatures are characterized by distinct immunophenotypes. *MEF2D*r ALL tends to lack CD10 surface marker while expressing CD38; whereas *ZNF384*r ALL exhibits lineage plasticity and may be found in approximately half of B/myeloid mixed-phenotype acute leukemia (MPAL) with frequent co-expression of myeloid markers (CD13 and CD33) and lack of CD10 expression [33, 34]. Considering the lineage aberrancy in a patient with MPAL, *ZNF384* fusion may represent a more reliable diagnostic biomarker rather than relying on the immunophenotype [9, 35].

Rare newly defined subgroups

Most recently, two different teams have described novel rare subgroups, totaling up to 23 B-ALL subtypes [11, 31]. The rarity of these subsets yields uncertain prognostic interpretation that will require further validation. First, fusions in the chromatin regulator *NUTM1,* described in about 1% of B-ALL, harbor a unique transcriptional signature and enriched among *KMT2A*-germline infant ALL cases [36]. Secondly, while *IKZF1* alterations can be encountered across different molecular subgroups in B-ALL, the missense mutation *IKZF1* N159Y reveals a strikingly distinct molecular signature. Finally, despite recurrent *IGH* rearrangements to multiple partners are often encountered in B-ALL, the rearrangement of *IGH* to *BCL2*, *MYC* and/or *BCL6* defines a new subgroup that is present mostly in adult ALL, accounting for 1% of them [11, 31]. Thus, the advent of NGS, in particular with the increasing utilization of whole transcriptome analysis or other clinical RNA-based fusion assays, now enables molecular profiling and classification for approximately 95% of all pediatric B-ALL [9, 27]. However, it is important to recognize that the prognostic significance of these rare molecular subgroups is limited by the paucity of cases and should be confirmed in large, prospective, multicenter clinical trials. It is unknown whether the prognostic impact of these gene alterations remains independently adverse in the context of modern MRD-directed treatment strategies.

T-lineage ALL

T-cell ALL (T-ALL) represents 15% of pediatric ALL and up to 25% of adult ALL [2, 37]. Its prognosis is historically considered inferior to that of B-ALL and remains a high-risk determinant in several ALL protocols. However, with contemporary intensive risk-adapted

treatment, the 5-year event-free survival (EFS) of T-ALL exceeds 85%, thus approximating that of HR B-ALL [8]. Unlike for B-ALL, the incorporation of molecular classification in T-ALL trials is hindered by the heterogeneity of genetic alterations found in T-ALL [8, 37]. Immunophenotyping classification was first used to identify the high-risk subgroup of early T-cell precursor (ETP) ALL which is characterized by lack of CD1a and CD8, dim CD5, and the expression of aberrant stem-cell or myeloid markers [38, 39]. About 12% of pediatric T-ALL is categorized as ETP leukemia and 17% in the highly similar group of near-ETP leukemia (identical phenotype but with an elevated CD5 expression). Compared to non-ETP leukemias, these two subgroups are more likely to be resistant to chemotherapy with high frequency of induction failure and MRD positivity [38, 40]. However, when stratified by MRD response, the outcome is similar between the different phenotypes and confirms that the prognostic impact of MRD prevails over the immunophenotype [41]. More recently, high-throughput genome sequencing of a large T-ALL cohort identified 106 putative gene alterations and partitioned into eight distinct molecular subgroups (**Figure 2**). The prognostic impact of the sentinel genetic alterations in T-ALL remains uncertain and did not outperform MRD-based risk classification [41, 42]. T-ALL molecular profiling is characterized by a large number of biologically relevant genomic alterations with 10 to 20 lesions in each individual leukemia [43]. Some anomalies are highly prevalent and occur in the vast majority of T-ALL. For example, activating *NOTCH1* mutations and *CDKN2A*/*CDKN2B* deletions are present in more that 50% and in up to 70% of T-ALL, respectively [42, 44, 45]. Alterations encountered in T-ALL can be subdivided into different signaling pathways: transcriptional regulation, NOTCH1 signaling, cell cycle control, kinase activation, epigenetic regulation, RNA processing, ribosomal function and ubiquitination [10, 42, 43]. Alterations in transcription factor regulation are nearly universal in T-ALL. T-cell receptor rearrangements, placing oncogenic transcription factor genes under the control of strong T-cell specific enhancers, define major T-ALL subtypes with remarkable transcription factor activation: *TLX1, LYL1, LMO2/LYL2, TLX3* and *NKX2-1*. Deregulation in the transcription factors *HOXA*, *LMO1/LMO2* and *TAL1* categorizes three other subclasses [42, 45]. Signaling pathway activation was observed in 65% of pediatric T-ALL affecting PI3K-AKT (28%), JAK-STAT (25%) and RAS (14%). PI3K-AKT pathway mutations predominate in the TAL1 subtype, while JAK-STAT and RAS pathway mutations are more common in *TLX1, TLX3* and *HOXA* subgroups. Interestingly, some genetic alterations are shared between B- and T-ALL, including

KMT2A rearrangements (10-15% of T-ALL), *ABL1* rearrangements, alterations in cell cycle genes (*CDKN2A*/*B*) and epigenetic regulators (*CREBBP*) [42]. The molecular classification partially correlates with the immunophenotypic subgroups. ETP leukemia is found to be enriched in *LMO2*/*LYL2*; near ETP leukemia in *TAL1* and *TLX3*-dysregulated subgroups; whereas non-ETP leukemia is associated with *TAL1* and *TLX1* deregulation [39, 42]. ETP ALL harbors recurrent activating mutations in JAK-STAT and RAS-MAPK signaling pathways; some of these mutations are also observed in acute myeloid leukemia. The gene expression profile of ETP ALL, as well as its immunophenotype and mutational landscape, share similarities with stem-cell and myeloid precursors, suggesting that ETP cells of origin may derive from a multipotent stem cell [39, 42, 46, 47].

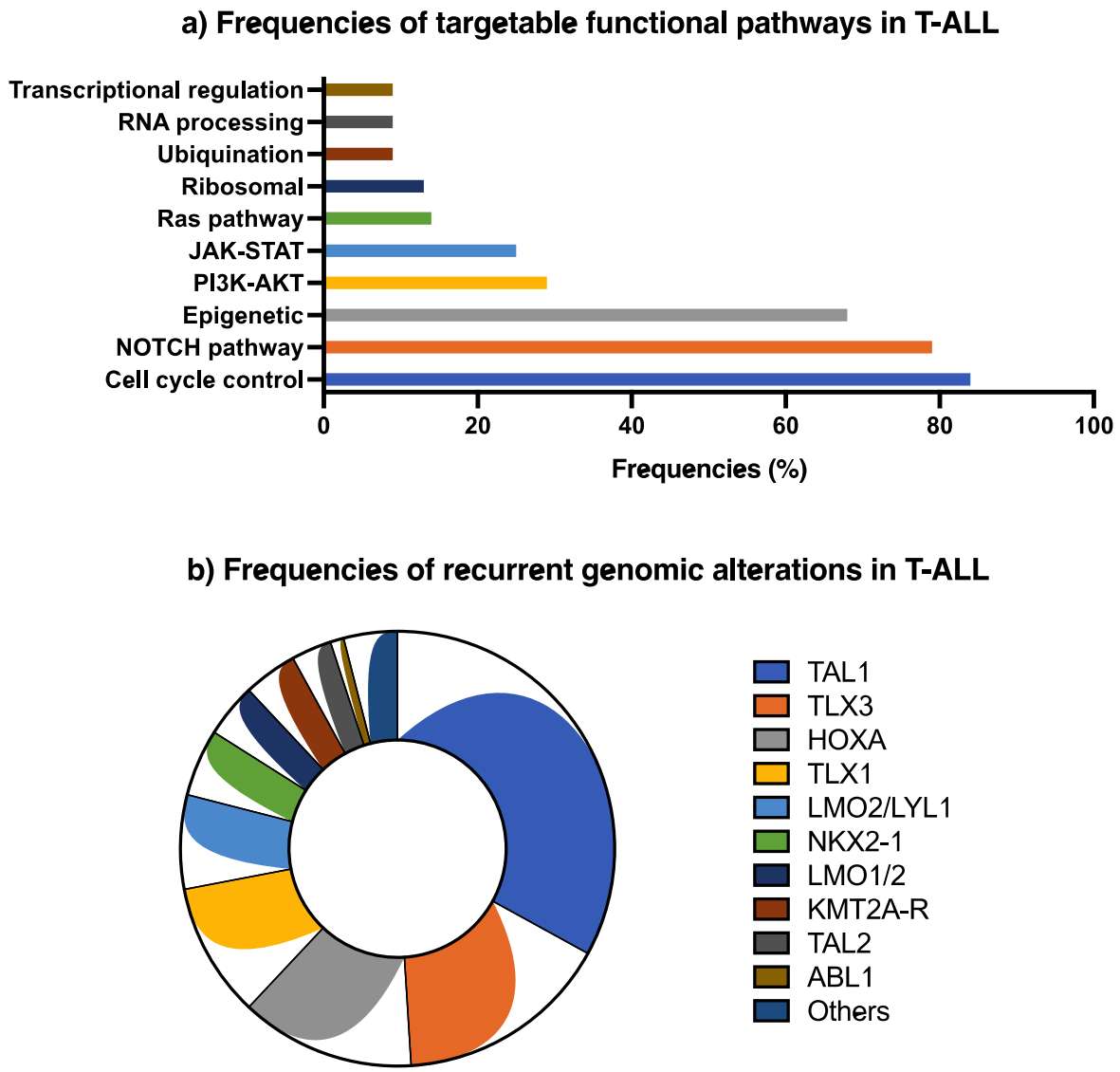


Figure 2. a) Targetable functional pathways and b) recurrent genomic alterations in T-lineage acute lymphoblastic leukemia. Frequencies are presented as percentages.

Relapsed ALL

Relapses occur in 10% to 20% of pediatric ALL following first-line therapy and remain one of the leading causes of cancer-related mortality in childhood [10, 48]. The genomic landscape of relapsed ALL has been explored by large-scale genome-wide studies involving matched diagnostic-relapsed leukemia samples. The mutational burden at relapse is increased compared to

that at diagnosis, with frequent acquisition of genetic alterations that were absent at initial presentation [49, 50]. The mutations present at diagnosis and relapse involve several similar pathways: RAS signaling (*NRAS*, *KRAS*, *PTPN11*, *FLT3*), JAK-STAT pathway (*ILR7*), NOTCH1 signaling (*NOTCH1*, *FBXW7*), transcription factors of lymphoid development (*IKZF1*, *ETV6*, *PAX5*), cell cycle control (*CDKN2A/B*, *TP53*) and epigenetic modulators (*KMD6A*) [49, 51]. Some of the mutations retain from diagnosis to relapse, others are volatile and can either be lost or gained at relapse. Volatile dynamics are more likely to be observed with *FLT3, JAK2* and *RAS* pathway mutations, while mutations of *IKZF1* and *CREBBP* are rarely lost at relapse when present at diagnosis [48, 52]. Most of the mutations identified at relapse may evolve from a subclonal population. Rarely, secondary leukemias may arise and do not share any genomic signature with the initial leukemia [49, 50, 52]. However, studies analyzing the clonal lineage with highly sensitive methods for mutation tracking were able to detect the ancestral clone, questioning the real prevalence of secondary leukemias. Even some relapses characterized by a lineage shift reveal mutational similarities between diagnosis and relapse, pointing to a unique clonal origin [35, 48, 49]. Recently, some mutations exclusive to relapsed ALL samples have been identified (*NCOR2*, *USH2A*, *NT5C2* and *PRPS1*), these mutations are never observed at diagnosis and suggest that they are therapy-induced [48, 53]. Similarly, a number of mutations enriched at relapse confer resistance to therapeutic agents commonly used in ALL and could have been selected during treatment. These mutations can alter sensitivity to glucocorticoids (*NR3C1*, *NR3C2*, *CREBBP*, *WHCS1*), purines analogs (*NT5C2*, *PRPS1*, *PRPS2*, *MSH2*, *MSH6*, *PMS2*), methotrexate (*FPGS*) and anthracycline/vincristine (*TP53*) [48, 51, 53-55]. The clonal evolution of these alterations may account for the different relapse patterns. For instance, early relapse arises from a minor subclone that survives and acquires a secondary resistant mutation during treatment, whereas very early relapse emerges from a pre-existing resistant clone [51].

INHERITED GENETIC VARIANTS IN PEDIATRIC ALL

Large scale genome analysis has also unraveled novel germline predisposition syndromes. Before genomic characterization, only a few mendelian diseases were associated with a higher incidence of childhood ALL, such as Down syndrome (DS), ataxia telangiectasia, Bloom syndrome and constitutional mismatch repair deficiency [56-58]. The children with DS have a 10 to 20-fold increased risk of developing ALL compared to the general population [57, 59]. DS-

ALL, unlike DS-related acute myeloid leukemia, occurs rarely in the first year of life and is associated with an inferior outcome in comparison to non-DS ALL. The lower survival can be attributed to a higher relapse rate and a susceptibility to chemotherapy in DS patients who display a heightened treatment-related toxicity. Nevertheless, the survival difference is not observed across different clinical trials and might be explained by the specific genomic landscape of DS-ALL [57, 60]. ALL from DS patients is less likely to carry favorable cytogenetic alterations, such as *ETV6-RUNX1* or hyperdiploidy, and is enriched in *CRLF2* rearrangements, in 50 to 60% of DS-ALL cases, with frequent *JAK2* mutations, a similar profile to what is encountered in Ph-like ALL [59-61]. However, in contrast to HR B-ALL without DS, the presence of *CRLF2* alterations in DS-ALL does not correlate with a worse prognosis, as reported by a large retrospective cohort comprising 317 DS-ALL patients treated on COG clinical trials between 2003 and 2016 [62].

New germline alterations have been recently discovered since the NGS advent. The germline variants conferring ALL susceptibility can be subdivided into two categories: 1) rare pathogenic germline variants with high penetrance (*TP53* and transcription factor genes *ETV6*, *PAX5* and *IKZF1*) found in families with high ALL incidence; 2) a growing number of common inherited variants with low penetrance detected via genome wide association studies (GWAS) (*ARID5B, CEBPE, CDKN2A/B, GATA3, PIP4K2A, TP63)* [56, 63-65]. Similar to somatic alterations in *IKZF1,* germline mutation alters IKAROS function and affects treatment sensitivity both *in vivo* and *in vitro* by reducing dexamethasone and dasatinib tyrosine kinase activity in leukemic cells [66]. Some of these inherited variants predispose to specific ALL molecular subgroups. Inherited *TP53* mutations, causing Li-Fraumeni syndrome, occur in about 50% of low-hypodiploid ALL [64]. Variants in *GATA3* are strongly associated with Ph-like ALL, while *TP63* variants preferably relate to the *ETV6-RUNX1* subgroup and *PIP4K2A* to the hyperdiploid ALL [65, 67-69]. Finally, GWAS has uncovered the association of inherited gene polymorphisms and drug sensitivity or toxicity and laid the foundation for clinical pharmacogenomics. The most significant are *TPMT* and *NUDT15* variants responsible for thiopurine-induced myelosuppression [65]. The prevalence of these variants follows ethnic variations; the former being more frequent in African descendent and the latter in people from East Asian or Hispanic origins. Heterozygous patients for these variants tolerate lower dose of thiopurine, approximately

60% to 70% of the usual dosing, while homozygous patients exhibit extreme mercaptopurine sensitivity and necessitate drastic dose reduction to 10% of the intended dose. Pre-emptive screening for these variants is now recommended for pharmacogenomics-based therapy adjustment [70].

TARGETED PRECISION MEDICINE OPPORTUNITIES

The most spectacular survival improvement for relapsed and refractory (R/R) ALL has been achieved with the recent advent of immunotherapy. Bispecific T-cell engager (BITE) antibody (e.g. blinatumomab), antibody-drug conjugates (e.g. inotuzumab ozogamicin), or cellular therapy (e.g. chimeric antigen receptor (CAR) T-cells), have shown impressive response in heavily pretreated R/R ALL [71-73]. Immunotherapy-based treatment strategies, targeting common ALL surface antigens, have the advantage of being agnostic to sentinel genetic alterations, and therefore, can be applied to a broader patient population [10]. Thus, there is still room for improvement since a non-negligible proportion of patients fail to respond or relapse after immunotherapy. We hereby review emergent molecularly targeted therapies and precision medicine opportunities in pediatric ALL with the potential for further survival improvement.

Ph^+ ALL

The success of tyrosine kinase inhibitor (TKI)-based treatment in Ph^+ ALL is certainly one of the most eloquent examples of precision oncology. Until the early 21st century, Ph^+ ALL, which concerns 3 to 5% of pediatric ALL, had a dismal outcome. Despite high-intensity chemotherapy followed by allogenic hematopoietic stem cell transplantation (HSCT) intensification in first complete remission, less than 50% of the patients were long-term survivors [74]. Following the remarkable clinical efficacy of the BCR-ABL TKI, imatinib, in chronic myeloid leukemia (CML), TKI became a therapeutic opportunity for Ph^+ ALL [8]. Clinical trials from the COG and the European EsPhALL consortia have demonstrated a robust survival benefit in Ph^+ ALL with the incorporation of imatinib combined to an intensive chemotherapy backbone, thus challenging the indication of HSCT in first remission in the TKI era. With imatinib-based regimens, almost 70% of Ph^+ALL patients can avoid HSCT and achieve durable remissions [75-78]. The second generation BCR-ABL1 TKI, dasatinib, is an alternative to imatinib in combination to intensive chemotherapy that has been tested in COG and EsPhALL non-randomized clinical trials. In these

studies, dasatinib did not contribute to further improve the clinical outcome, showing a similar efficacy to historical imatinib-based regimens [79, 80]. However, earlier introduction of protracted TKI administration by mid-induction appears to increase the achievement of post-induction MRD negativity, then reducing the need of HSCT consolidation in first remission. The Chinese Children's Cancer Group (CCCG) ALL-2015 phase 3 randomized trial compared the combination of either dasatinib or imatinib to the St. Jude Total Therapy chemotherapy backbone and suggested dasatinib's superiority over imatinib with regards to EFS and OS. This result needs to be confirmed as the median follow-up remains relatively short. In addition, the outcomes in the imatinib arm were unexpectedly inferior compared to those of prior imatinib-based regimens in COG or EsPhALL trials and the TKI dosage used was different than prior studies [81]. The promising success of TKI and chemotherapy combination in the treatment of Ph^+ALL has established a new treatment paradigm for ALL in the molecular era, nourishing the hope of new therapeutic opportunities by targeting novel oncogenic drivers in ALL. Recently, the combination of TKI and immunotherapy further expands the precision medicine paradigm in Ph^+ALL. The chemotherapy-free phase 2 D-ALBA study demonstrated early promising results in adults with newly-diagnosed Ph^+ ALL. The treatment consists of 85-day induction phase combining dasatinib and glucocorticoids followed by a consolidation with 2 cycles or more of the CD3/CD19 bi-specific antibody, blinatumomab, in association with dasatinib. Interestingly, after induction therapy with dasatinib and glucocorticoids only, 98% of the patients achieved a complete response, and 29% a molecular response. After 2 cycles of blinatumomab and dasatinib consolidation, the molecular response rate exceeded 60% [82].

Ph-like ALL

The clinical and biologic similarities between Ph-like ALL and Ph^+ ALL provide the rationale to model tailored TKI-based therapy in Ph-like ALL with an anticipated efficacy similar to that observed in Ph^+ ALL. *In vitro* and *in vivo* evidence have reinforced the putative sensitivity of Ph-like ALL to selected TKIs by demonstrating activity of ABL inhibitors, imatinib or dasatinib, and JAK inhibitor, ruxolitinib, for those harboring ABL-class fusions and JAK-STAT pathway mutations, respectively [19, 20, 83-85]. There is a growing body of clinical evidence from case reports or small patient series to confirm the preclinical efficacy of ABL and JAK inhibitors in Ph-like ALL [86-88]. Preclinical and clinical reports have also demonstrated the sensitivity of

NTRK inhibitor, larotrectinib, or ALK inhibitor, crizotinib, for the rare *ETV6-NTRK3* Ph-like ALL [19, 89, 90]. Recently, mutations conferring resistance to imatinib and dasatinib have been identified in relapsed *EBF1-PDGFRB* Ph-like ALL, raising the necessity to monitor kinase domain mutations over the course of therapy to guide TKI selection [91]. Thanks to the progress in genomic characterization of ALL, Ph-like ALL has become a new paradigm of tailored precision medicine, but prospective clinical trials are much needed to confirm the benefit of TKI and chemotherapy in Ph-like ALL. Several ongoing clinical trials should shortly answer some of these urgent questions (Total XVII (NCT03117751); AALL1131 (NCT01406756); AALL1521 (NCT02723994) (**Table 1**).

Table 1. Precision medicine opportunities in pediatric acute lymphoblastic leukemia

ALL subtypes	Therapies	Preclinical – Single patient experience	Clinical trials	Phase
Ph$^+$ ALL	**Imatinib**		**AALL0031 (NCT00022737)** **EsPhALL2010 (NCT00287105)** **CCCG-ALL-2015** **EsPhALL 2017/COG AALL1631 (NCT03007147)**	**3** **2** **3** **3**
	Dasatinib		**AALL0622 (NCT00720109)** **AALL1122 (NCT01460160)** **CCCG-ALL-2015**	**2** **2** **3**
	Ponatinib		**NCT04501614**	**1/2**
		Rexinoids [92]		
		FAK inhibitors [93]		
Ph-like ALL **- *with ABL-class fusions***	**Dasatinib**		**AALL1131 (NCT02883049)** **Total Therapy XVII (NCT03117751)**	**3** **3** **3**

			DFCI ALL 16-001 (NCT03020030)	
- with JAK-STAT pathway lesions	**Ruxolitinib**		**AALL1521 (NCT02723994)** **Total Therapy XVII (NCT03117751)**	**2** **3**
- with NTRK fusions	**Larotrectinib**		**ADVL1823 (NCT03834961)**	**2**
		PI3K/AKT/mTOR inhibitors [85]		
KMT2A-R ALL	**Lestaurtinib**		**AALL0631 (NCT00557193)**	**3**
	Azacitidine		**AALL15P1 (NCT02828358)**	**2**
	Vorinostat/ Bortezomib		**TINI (NCT02553460)**	**1/2**
	Menin inhibitors		**NCT04811560** (adults only)	**1**
	DOT1L inhibitors		**NCT02141828**	**1**
	Venetoclax		**NCT03826992**	**1**
Hypodiploid ALL		**Venetoclax [94]**		
ZNF384-R ALL		**FLT3 inhibitors [95]**		
MEF2D-R ALL		**HDAC inhibitors [33]**		
T-ALL	**Venetoclax**		**NCT00501826**	**2**
- NOTCH pathway mutations		**Y-secretase inhibitors, Soluble notch proteins, Mastermind inhibiting peptides [96]**		
- JAK-STAT	**Ruxolitinib**		**Total Therapy XVII**	**3**

mutations **-ETP ALL**			**(NCT03117751)**	
- ***PI3K/AKT/mTOR pathway mutations***	**Everolimus**	**PI3K/AKT/mTOR inhibitors [97]**	**DFCI 11-237 (NCT01523977)**	**1**
		Farnesyltransferase inhibitors [98]		
- ***Ubiquination***	**Bortezomib** **Carfilzomib**		**AALL1231 (NCT02112916)** **Total Therapy XVII (NCT03117751)** **CFZ008 (NCT02303821)**	**3** **3** **1**
B and T-ALL **-** ***Cell cycle control***	**Palbociclib** **Ribociclib**		**AINV18P1 (NCT03792256)** **NCT03740334**	**1** **1**
- ***Ras pathway mutations***	**Selumetinib**		**NCT03705507**	**1/2**

ALL: acute lymphoblastic leukemia, *ETP*: early T-cell precursor, HDACi: HDAC inhibitor, *KMT2A-R*: *KMT2A* rearranged, *MEF2D-R*: *MEF2D* rearranged, *Ph+*: Philadelphia positive, *T-ALL*: T-cell acute lymphoblastic leukemia, *ZNF384-R*: *ZNF384* rearranged

Proapoptotic targeted therapy

Proapoptotic agents, acting as cell death inducers by overcoming chemoresistance, represent another promising therapeutic opportunity for ALL [99]. *KMT2A* rearrangements, frequently observed in infant ALL, are known to confer resistance to apoptosis, emphasizing the interest of apoptotic inducer for this subgroup [100, 101]. A study on xenografts confirmed the activity of the selective BCL-2 inhibitor, venetoclax, for *KMT2A*r ALL models, while other ALL subtypes escaped venetoclax inhibition by activating the bcl-x pathway [99]. More recently, venetoclax appeared to provide *in vitro* and *in vivo* efficacy for controlling the leukemic burden in hypodiploid ALL, in *TCF3-HLF* ALL and in ETP ALL [94, 102, 103]. The potential activity in T-ALL has been reinforced by case reports of venetoclax activity, in association with decitabine

or bortezomib [104, 105]. A pediatric phase 1 clinical trial of venetoclax with chemotherapy combination showed good tolerance to the regimen and encouraging clinical activity in ALL patients [106]. The addition of the BCL-2/BCL-X_L/BCL-W, navitoclax, to venetoclax and chemotherapy seems to enhance the clinical activity of venetoclax by overcoming escape pathways. A phase 1 study with this combination reported a promising activity of 86% of response in pediatric relapsed/refractory ALL patients, among whom 56% achieved negative MRD [107]. Targeting proapoptotic pathway offers an optimistic outlook in the landscape of R/R ALL treatment, but the best combination and ideal target population have yet to be determined.

Therapeutic opportunities for rare molecular subgroups

The modern genomic taxonomy of ALL identified some therapeutic susceptibility in rare molecular subsets, but clinical validation is required. The extensive genomic characterization by whole genome and transcriptome analysis of a *ZNF384*-rearranged ALL patient revealed a highly aberrant *FLT3* overexpression. This patient, presenting with refractory disease, happened to be highly sensitive to the FLT3 inhibitor, sunitinib, leading to deep MRD-negative response and long-term survival [95]. This case illustrates how the combination of genome and transcriptome analysis can lead to the identification of unsuspected therapeutic vulnerabilities. Another example is the constant overexpression of the histone deacetylase *HDAC9*, a transcriptional target of MEF2D, observed in *MEF2D*-rearranged ALL. Xenograft models were used to test the therapeutic vulnerability of *MEF2D*r ALL to the HDAC inhibitor, panabinostat, and showed exquisite *in vivo* sensitivity [33]. No clinical experience has been reported yet. Finally, *IKZF1* inactivating mutations induce *in vitro* stem cell and adhesive properties and alter the response to dasatinib in *BCR-ABL1* mouse models. Retinoids and focal adhesion kinase (FAK) inhibitors have the ability to reverse these mechanisms, thus restoring dasatinib sensitivity [92, 93]. Retinoids and FAK inhibitors constitute potential therapeutic avenues that can be explored for *IKZF1*-mutated Ph^+ ALL and Ph-like ALL, and for the *IKZF1* N159Y subgroup.

A major challenge for the translation and implementation of the modern ALL taxonomy into pragmatic clinical algorithms is the limited access to comprehensive NGS platforms and their relatively long turnaround time, making it challenging for real time patient's care. Nevertheless,

a number of clinically-validated NGS assays are increasingly available and being incorporated into frontline ALL trials. For example, the Rapid Heme Panel, a DNA-based NGS diagnostic assay currently used in the DFCI ALL 16-001 study for the detection of sequence mutations and CNAs, is able to deliver results within 10 days for risk stratification [108]. Gene expression profiling for the identification of Ph-like ALL has now been largely replaced by the TaqMan low-density array (LDA) microfluidic card measuring the expression of 8 or 15-gene panels to determine the Ph-like ALL signature. This LDA-based approach has provided a rapid and cost-effective screening modality to identify patients with probable Ph-like ALL (LDA-positive) who require further detailed genomic characterization. To identify targetable kinase-activating alterations, the ArcherDx FusionPlex Heme panel uses anchored multiplex PCR-based enrichment with the ability to detect novel fusions involving 87 genes associated with hematologic malignancies [109]. The AIEOP-BFM consortium is now incorporating array comparative genomic hybridization to identify the IKZF1^{plus} profile and panel-based RNA sequencing to detect targetable gene fusions for upfront risk stratification in their frontline ALL trial [110]. At the level of a single center, the St. Jude Children's Research Hospital attests that a full DNA- and RNA-based sequencing approach is feasible within 4 weeks and suitable for integration into patient's real-time management [111]. The modernization of sequencing technologies and the development of standardized bioinformatic pipelines for timely data analysis should facilitate routine clinical implementation of genomic profiling for pediatric ALL.

CONCLUSION

Advances of high-throughput sequencing technologies redefine the genomic portrait of ALL and modernize ALL classification, with only a marginal proportion of patients remaining unclassified in the current molecular landscape. Beside Ph$^+$ and Ph-like ALL for whom robust preclinical and clinical evidence supports the prospective assessment of TKI and chemotherapy in frontline trials, a substantial proportion of molecular subsets, particularly those associated with unfavorable outcomes, still lacks access to key therapeutic targets and relevant precision medicine trials. Continuous efforts in elucidating the functional mechanisms underlying the subtype-defining alterations in ALL is essential to expand the spectrum of novel targeted therapies. Nevertheless, the development of new therapeutic options and consequently the design of clinical trials are hindered by the growing number of molecular subsets, each accounting for a small proportion of

ALL. Large international clinical trials are therefore critical to explore innovative treatments combining chemo-, immuno- and targeted therapies, with the objectives of improving survival in high-risk subtypes and reducing toxicity in low-risk ALL. The current molecular era of ALL present unique challenges but also offers exciting opportunities for paradigm-shifting therapies.

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Authors' contributions

RS and THT performed the literature review and wrote the manuscript. Both authors contributed equally to this work.

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The authors declare no conflicts of interest.

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