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ACUTE LYMPHOCYTIC LEUKEMIAS (M KENG AND F EL CHAER, SECTION EDITORS)

Application of Next-Generation Sequencing-Based Mutational Profiling in Acute Lymphoblastic Leukemia

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Abstract

Purpose of Review Recent efforts to characterize hematologic cancers with genetic and molecular detail have largely relied on mutational profiling via next-generation sequencing (NGS). The application of NGS-guided disease prognostication and clinical decision making requires a basic understanding of sequencing advantages, pitfalls, and areas where clinical care might be enhanced by the knowledge generated. This article identifies avenues within the landscape of adult acute lymphoblastic leukemia (ALL) where mutational data hold the opportunity to enhance understanding of disease biology and patient care.

Recent Findings NGS-based assessment of measurable residual disease (MRD) after ALL treatment allows for a sensitive and specific molecular survey that is at least comparable, if not superior, to existing techniques. Mutational assessment by NGS has unraveled complex signaling networks that drive pathogenesis of T-cell ALL. Sequencing of patients with familial clustering of ALL has also identified novel germline mutations whose inheritance predisposes to disease development in successive generations.

Summary While NGS-based assessment of hematopoietic malignancies often provides actionable information to clinicians, patients with acute lymphoblastic leukemia are left underserved due to a lack of disease classification and prognostication schema that integrate molecular data. Ongoing research is positioned to enrich the molecular toolbox available to clinicians caring for adult ALL patients and deliver new insights to guide therapeutic selection, monitor clinical response, and detect relapse.

Keywords Acute lymphoblastic leukemia · Next-generation sequencing · Mutational profiling · Measurable residual disease · Germline predisposition

Introduction

With more than 6000 new diagnoses expected in the USA each year, acute lymphoblastic leukemia (ALL) is the second most common acute leukemia of adults (https://seer.cancer.gov/statfacts/html/alyl.html). The disease is characterized by the proliferation of lymphoid precursor cells and propagated by the acquisition of chromosomal alterations and driver mutations in critical genes. ALL is the most common malignancy of childhood, where cure rates currently exceed 90% with multi-agent chemotherapy [1]. By contrast, the success of chemotherapy in adult ALL is less dramatic, partly due to intrinsically more adverse risk disease in this population, but also due to patient-specific factors such as older age and comorbidity which limit the capacity of older ALL patients to tolerate multi-agent chemotherapy. Adult-specific multi-agent chemotherapeutic regimens have historically produced cure rates between 40 and 50% despite high rates of initial complete remission, although the more routine adoption of truly pediatric regimens for the younger adult population (those < 40 years) appears to have resulted in a marked improvement in outcome [2, 3]. Despite some successes, relapse remains a significant problem and strategies aimed to detect, characterize, and reduce the incidence of relapse are an area of active investigation.

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ALL manifests without pathognomonic clinical features and patient presentations are often characterized by nonspecific symptoms including fevers, fatigue, diaphoresis, bone pain, or bleeding complications. The diagnostic workup entails a thorough history and physical exam, laboratory studies, imaging to survey for extramedullary involvement, lumbar puncture with analysis of the cerebrospinal fluid, and bone marrow aspiration and biopsy. Disease subtype classification and risk stratification is accomplished by morphologic examination, cytogenetic analysis, and flow cytometry immunophenotyping [4]. The 2016 revision to the World Health Organization classification schema further defined two new categories for T-lymphoblastic (T-ALL) and B-lymphoblastic ALL (B-ALL), and describes provisional entities within the B-ALL categorization including B-ALL with intrachromosomal amplification of chromosome 21 and B-ALL with a gene expression profile resembling BCR-ABL + ALL (Ph-like ALL) [5].

Molecular profiling for patients with ALL remains an area of active research. When compared to solid tumors, hematologic malignancies are relatively bland with respect to the number and diversity of mutational events [6]. In recent years, the therapeutic toolbox for solid tumors has morphed from one heavily reliant on combination chemotherapy regimens to one rooted in the use of targeted therapies directed at oncogenic driver mutations. The pace of targeted therapy development for hematologic malignancies has generally lagged behind, and, until recently, disproportionally favored chronic myeloid and lymphoid leukemias for which imatinib and ibrutinib (among others) have significantly improved outcomes. Since 2017, several new treatments have also been FDA-approved for the treatment of acute myeloid leukemia (AML). Use of many of these therapies relies on the detection of mutations in specific targetable genes for which novel therapies have been designed. Based on guidelines from the European Leukemia Net and the National Comprehensive Cancer Network, AML patients should undergo comprehensive molecular sequencing of genes with immediate implications for targeted therapeutics, including c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA, IDH1/2, RUNX1, ASXL1, and TP53 [7, 8]. While gene-by-gene, PCR-based strategies are feasible, testing with larger panels using next-generation sequencing (NGS) is well-validated and could potentially identify additional informative mutations [9]. For these reasons, the European Leukemia Net 2017 update supports panel-based NGS for molecular prognostication and target identification in patients with myeloid leukemia.

According to the National Comprehensive Cancer Network guidelines for both adult and pediatric ALL, upfront NGS-based mutational workup is not uniformly recommended [10, 11]. This is likely due to the heterogeneity that exists among sequencing methodologies, a lack of harmonization regarding a "minimum" set of target genes to be included in any given assay, and center-dependent factors such as operator expertise and laboratory limitations. However, de facto practices at many major academic centers have led to the collection of mutational data and guided strategies for the provision of targeted therapies in either compassionate use settings or through formal clinical protocols. A model for how acquisition of molecular data has informed treatment selection for Ph-like ALL is depicted in Fig. 1. Several articles in the literature have also comprehensively summarized classification schema, prognosticating factors, available treatments, and considerations surrounding allogeneic stem cell transplantation in patients with ALL [12–14]. In this a review, we consider emerging uses for NGS-based mutational profiling in patients with acute lymphoblastic leukemia, with a focus on adult ALL mutational profiling. We will highlight three exciting avenues within the ALL landscape that have been delineated by NGS approaches and that hold implications for disease prognostication and clinical decision making: (1) molecular measurable residual disease testing, (2) disentangling the complicated biology of T-ALL, and (3) ALL germline predisposition syndromes.

Molecular MRD in ALL

Leukemic relapse stems from the persistence of malignant cells after completion of treatment that are capable of propagating the return of clinically overt disease. Measurable residual disease (MRD) is the term used to describe the detection of small populations of clinically relevant leukemia phenotypes capable of resulting in leukemic relapse. Detection of MRD has been shown to have powerful prognostic implications in a variety of clinical contexts. Techniques to characterize and quantitate MRD in ALL were first widely validated and used in pediatric ALL cohorts, and eventually, more data in the adult population has allowed MRD to gain broad adoption across the spectrum of age. MRD testing can be achieved using a variety of techniques including multiparameter flow cytometry (MFC), real-time quantitative polymerase chain reaction (PCR), as well as NGS. Regardless of the modality used (discussed in detail below), large meta-analyses with cumulative totals including thousands of individual ALL patients have demonstrated markedly superior outcomes in terms of event-free and overall survival for those who attain states of MRD negativity (MRD −) compared to individuals who harbor detectable disease (MRD +) at the end of induction or consolidation [15]. The most straightforward approach for measuring MRD utilizes highly sensitive real-time quantitative PCR techniques with primers that probe for regions of the rearranged immunoglobulin and T-cell receptor genes in cells that comprise the ALL clone. MFC uses lasers to detect optically active antibodies

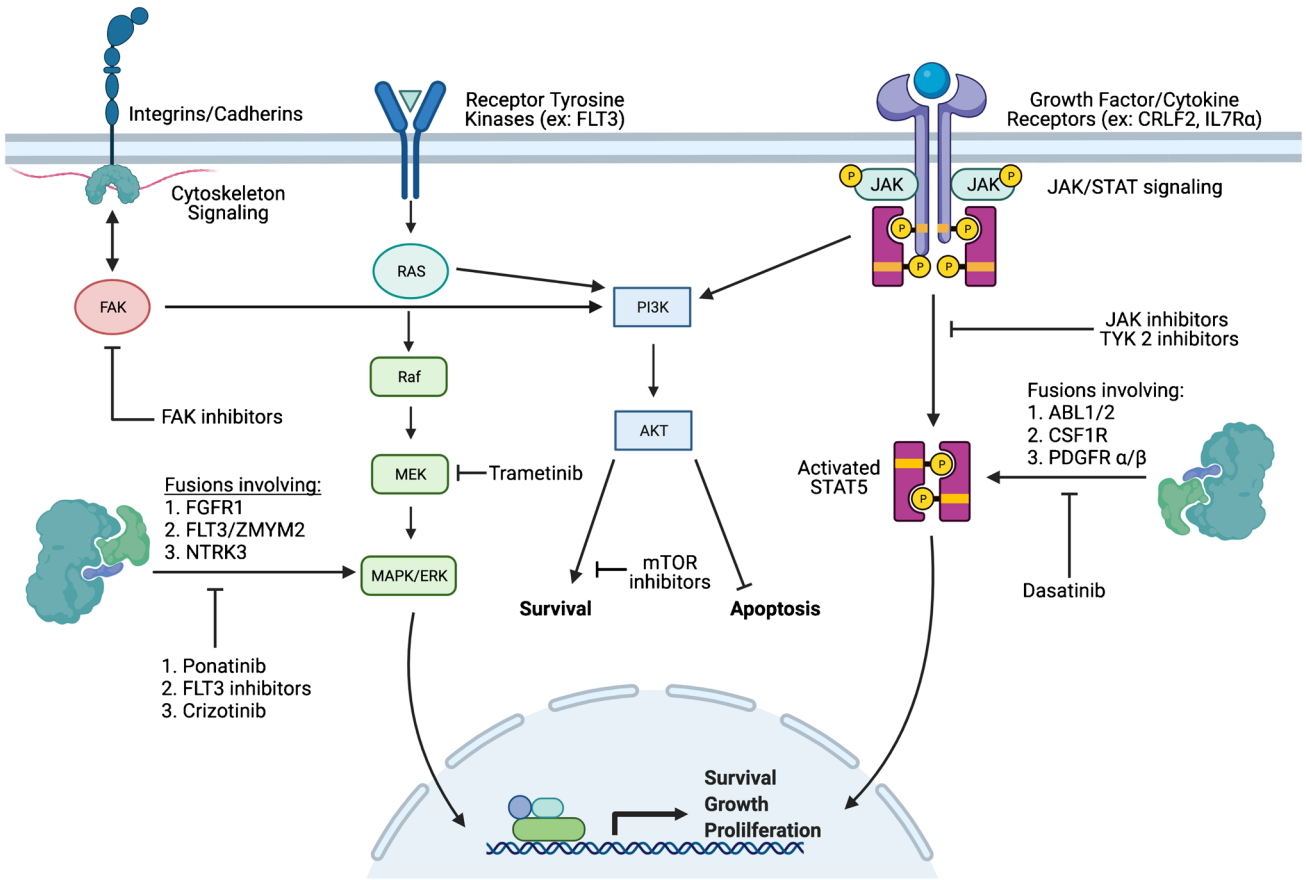

Fig. 1 Targeted kinase inhibition in Philadelphia chromosome (Ph)-like acute lymphoblastic leukemia. Common signaling pathways in Ph-like ALL can be categorized by (1) activating lesions in JAK-STAT pathways, including CRLF2, IL7R, EPOR rearrangements, JAK mutations, and SH2B3 deletion/mutation (not shown); (2) oncogenic fusion proteins involving ABL-class tyrosine kinases, and (3) other rearrangements involving kinases such as FLT3, FGFR1, and NTRK3 which propogate survival and proliferation of malignant cells via STAT-mediated Ras/PI3K predominant signaling. A rational approach to targeted kinase inhibition is shown based on a review of the literature [71–77]. Of note, Ras pathway mutations are not specific to Ph-like ALL and are present in other ALL subtypes. For simplicity and emphasis on therapeutic targets, kinase cascades in the pathways shown have been condensed and certain intermediate proteins omitted. Image created with BioRender

attached to cell surfaces and define a leukemia-associated immunophenotype (LAIP). An LAIP can be identified at the time of initial diagnosis and can therefore allow definition of an MRD population for a majority of both B-ALL and T-ALL patients. The sensitivity of MFC varies, with early 4–6 multicolor modalities possessing a limit of detection of 1 abnormal cell in a background of 10^3 or 10^4 normal cells. The newer 8–10 multicolor flow cytometry techniques can provide improved sensitivity on the order of 1 abnormal cell in a background of 10^5 normal cells [16, 17]. The major advantage to MFC-based MRD assessment over other techniques is its wide application; about 90% of patients can be assigned a LAIP at the time of first presentation. However, a critical limitation of MFC-based MRD is the impact of therapy-associated changes in clonal composition, which can result in phenotypic shifts in both non-leukemic cells and in the LAIP-associated MRD populations. Such changes can lead to both false positive and false negative reports for MRD status at the time of repeat evaluation and create a considerable clinical conundrum for patients and caregivers upon repeat survey [18].

As use of NGS-guided molecular assessment of both benign and malignant hematologic disorders continues to win favor [19], the optimization and clinical integration of NGS-driven MRD in ALL is underway [20]. Massively parallel, clinical NGS can be employed through numerous iterations, including targeted exon or "hotspot" sequencing, whole genome or whole exon sequencing, and transcriptome-focused methods via RNA sequencing. The sensitivity of NGS-based MRD assessments can range from 1 in 10^5 or 10^6 depending on the amount of input genetic material and the bioinformatic analysis technique employed. As with other NGS applications, NGS-based MRD is attractive due to its scalability and high throughput via automated wet lab

procedures reducing human "hands-on" time [21]. Additionally, with new "barcoding" techniques using unique molecular indices to watermark individual nucleic acid strands, samples from multiple patients or clinical timepoints can be pooled into single sequencing reaction without worry of contamination [22].

In the real world, targeted sequencing approaches often take the form of commercially available panels and includes genes that, when mutated, offer actionable prognostic or therapeutic information. These modalities, employed upfront at diagnosis, are now commonplace. The design and analysis of NGS MRD assays are drastically different depending on whether they are intended for myeloid or lymphoid malignancies. AML, for example, is a mutationally heterogeneous, polyclonal disease whose clonal architecture changes in response to therapy [23]. As a result, NGS-based AML MRD panels need to cover a large collection of relevant target genes in order to assign a mutational profile in each patient to be tracked longitudinally over time [24]. Genes encoding cell surface proteins that serve as therapy targets are generally poor MRD markers. For example, molecular analysis of patients enrolled in the landmark RATIFY trial establishing midostaurin as an effective treatment for *FLT3* mutant AML found that 46% of patients relapsed with FLT3 negative clones, emphasizing the lack of utility for *FLT3* mutational events in the context of MRD monitoring [25].

By contrast, NGS-guided MRD in the context of lymphoid malignancies is more straightforward. Because ALL demonstrates a lower degree of clonal heterogeneity, sequencing libraries can be easily constructed by targeting regions flanking the immunoglobulin (Ig) or T-cell receptor loci (TCR), functionally providing a digital readout of traditional PCR techniques. Junctional rearrangement sequences (V-D-J domains) can be detected in ~ 95% of ALL clones from patients, and this relative uniformity of sequence reads makes MRD detection possible for a majority of patients with ALL [26]. Because of the monoclonal nature of malignant lymphoid populations, a collaborative involving several European research groups with expertise in ALL MRD assay design, known as The EuroClonality-NGS Consortium (www.euroclonalityngs.org), formed in order to validate and standardize NGS assays in ALL. Their methodological guidelines for NGS-based ALL MRD were published in 2019, providing a harmonized benchmark for future research in the field. [27] Accordingly, the US FDA has authorized use of a commercial product known as the clonoSEQ Assay (Adaptive Biotechnologies, Seattle WA), a multiplexed PCR and NGS assay to identify and quantify MRD in B-cell malignancies (https://www.ajmc.com/view/fda-clears-cloneseq-to-detect-mrd-in-chronic-lymphocytic-leukemia). More recently, reports comparing the properties and utility of NGS vs. flow cytometry-based MRD in cohorts of ALL patients have favored strategies based upon NGS. The pediatric ALL literature is replete with examples of NGS-augmented MRD detection and clinical decision making, including robust data for patients with both B-ALL and T-ALL [28, 29]. Table 1 provides an overview on the relatively small body of literature using NGS-based MRD assays in the adult ALL patient population.

The concept of MRD is primarily one that centers around practical "actionability." Namely, is MRD prophecy, or does it represent a modifiable risk factor? Large meta-analyses in both adult and pediatric populations have underscored the importance of achieving MRD negativity in ALL [30, 31]. An early trial from Germany demonstrated the potential to employ blinatumomab, a bispecific T cell-engager (BiTE) antibody that recognizes CD19 on one side and CD3 on the other, facilitating enhanced T- cell recognition of malignant B-lymphoid residual disease, as a salvage therapy to eliminate residual disease after induction or consolidation. Of 21 patients treated, 16 became MRD −, yielding a response rate of 80%. All MRD responses occurred by the end of the first cycle of blinatumomab therapy. Follow-up at 33 months revealed that 61% of MRD responders avoided disease recurrence. While blinatumomab first gained FDA approval for use in Philadelphia-chromosome negative (Ph −) relapsed/refractory B-ALL in 2014 based on the results depicting a 43% response rate [32], follow-up trials in Philadelphia-chromosome positive (Ph +) and pediatric cohorts enabled the expansion of FDA approval to a wider patient population [33, 34]. Subsequently, a pivotal trial from the German-Austrian group using blinatumomab to facilitate clearance of MRD + disease studied 116 patients with B-ALL who had achieved CR but who remained MRD + when assessed by PCR or flow cytometry. After treatment with blinatumomab, 78% of patients became MRD −. Those who became MRD − with treatment had a relapse-free survival of 23.6 months compared to 5.7 months for those who remained MRD +, with respective overall survivals of 38.9 months and 12.5 months [35]. This led to FDA approval of blinatumomab in early 2018 for ALL with MRD + and notably was the first drug to gain approval on the basis of MRD status [36].

An additional antibody-based therapy known as inotuzumab ozogamicin, which targets CD22 on B-cells, has also yielded promising results both for patients with relapsed/refractory ALL, and specifically for patients with MRD +. The antibody–drug conjugate was approved after a 2017 phase III trial in adults with either Ph + or Ph − relapsed/refractory ALL achieved a CR rate of 80% vs. 29.4% who achieved CR in an identically sized control arm who received standard intensive chemotherapy [37]. As with blinatumomab, patients receiving inotuzumab ozogamicin also achieved significantly higher rates of MRD negativity (78%) than the control group 28%.

Table 1 Studies of next-generation sequencing-based measurable residual disease in adult acute lymphoblastic leukemia. Data and protocols shown include studies that were not exclusively executed for childhood ALL, which greatly outnumber adult-focused trials. Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; Ig, immunoglobulin; TCR, T-cell receptor; BM, bone marrow; SCT, stem cell transplant; MFC, multiparameter flow cytometry; NGS, next-generation sequencing; MRD, measurable residual disease; PB, peripheral blood; MCL, mantle cell lymphoma; MM, multiple myeloma; qPCR, quantitative polymerase chain reaction; TBI, total body irradiation

Population age (years)	Notes on patients analyzed	MRD methodology	Summary of findings	Reference
18–64	32 pts with B-ALL, 29/32 with one identifiable clonal Ig sequence found; specimens were predominately BM ($n=61$) over PB ($n=5$); all pts were pre-SCT treated on a double-induction chemotherapy trial	MFC, NGS	MRD with MFC and NGS showed 82% concordance; in 17% of cases, MRD was detectable by NGS but not MFC; NGS sensitive to detect low residual leukemic burden in PB samples	[71]
15 and older	55 pts with varied lymphoid malignancies selected for study, 15 ALL, 30 MCL, 10 MM; 26 samples from 15 ALL patients underwent NGS and qPCR-based MRD assessment	qPCR, NGS	Both PCR and NGS were able to identify clonal populations in specimens studied; both modalities consistently reached sensitivity of 10^{-5} and were concordant in 80% of cases	[72]
16–67	42 adult ALL pts who underwent SCT were considered, 29 included for study; 27 of 29 pts had 1 or more clonal Ig/TCR sequences suitable for MRD quantification	NGS	Pre-SCT MRD + > 10^{-4} HCT predicted post-HCT relapse (hazard ratio = 7.7); post-SCT MRD + > 10^{-6} had 100% positive predictive value for relapse with median lead time of 89 days (hazard ratio = 14)	[73]
1–25	NCT03509961	NGS	Phase II pilot trial to estimate survival after a non-TBI based SCT conditioning regimen in B-ALL pts who are shown to negative for MRD by NGS	[74]

From the trials mentioned above, a logical question might arise about the utility of stem cell transplantation in the setting of MRD status for patients who received either blinatumomab or inotuzumab ozogamicin. As noted by Kantarjian and colleagues, inotuzumab ozogamicin enabled 41% of patients to proceed to transplant while only 11% of patients in the standard of care arm received an allograft [37]. Thus, inotuzumab ozogamicin may reasonably be utilized as a bridge to transplant strategy. Also, roughly half of the patients in pivotal blinatumomab trials mentioned above proceeded to transplant [38]. While MRD status appears to be among the most important prognostic factors in determining overall survival in ALL [31, 39, 40], provocative questions arise regarding the potential for agents like blinatumomab or inotuzumab ozogamicin to spare patients the morbidity and mortality of transplant if MRD − status is achieved. Although controversial, it is generally accepted that patients with detectable MRD would derive benefit from transplantation [36, 41]. More recently, the CALBG 10,403 study, which in short, demonstrated the benefit of treating adult ALL with pediatric-inspired regimens, also revealed that patients who achieve MRD − after induction performed well without an allogeneic transplant [3]. What is less clear is whether patients who might convert from MRD + to MRD − states with agents such as blinatumomab or inotuzumab ozogamicin could safely forego transplantation and enjoy similar rates of relapse-free and overall survival. An ongoing Alliance Study A41501 (https://clinicaltrials.gov/ct2/show/NCT03150693) seeks to evaluate the addition of novel agents such as inotuzumab ozogamicin to the CALGB 10,403 chemotherapeutic backbone. If a higher proportion of patients are able to attain MRD −, perhaps more individuals could avoid the rigors of transplant but derive similar survival benefits.

Unraveling Consequential Pathways in T-ALL

T-ALL comprises about 25% of cases in adults and is generally associated with poorer outcomes, likely due to the inherent biology of the disease and a smaller selection of novel therapies directed against T-cells as opposed B-cells [12]. For example, popular agents in the MRD + setting, such as blinatumomab, work by promoting T-cell directed killing of B-cells, and will obviously not be effective when the target cell population is not comprised of B lymphoblasts [36]. Recently, additional consideration has been given to defining oncogenic genotypes and delineating their effects on the pathogenesis of T-ALL. Recognized T-ALL oncogenic pathways include constitutive activation of the *NOTCH1* circuit by a variety of *NOTCH1/FBXW7* mutations, as well as pro-proliferative *RAS/RAF/MEK* and *PTEN/Akt/mTOR* mutations. Detection of *NOTCH1/FBXW7* mutations in

the absence of *RAS* or *PTEN* variants predicts a favorable outcome and is found in almost 50% of adult T-ALL [42]. Conversely, the absence of *NOTCH1/FBXW7* or the presence of mutations in the *RAS/PTEN* pathway is associated with a poorer prognosis. In combination with MRD-based assessment, this additional layer of independent prognostic data may help to guide clinicians in clinical decision making, such as whether stem cell transplant should be pursued in first complete remission [30, 43].

Recognition of specific disease subsets, such as the early thymic precursor (ETP) ALL molecular subgroup, is another example of the way in which NGS characterization can enhance understanding of disease biology and outcomes. ETP ALL is a subset of T-ALL originally discovered in pediatric patients but subsequently also described in adults with an analogous transcriptional landscape. It has a characteristic immunophenotype demonstrating absent/nearly absent T-lymphocyte markers and positivity for at least one hematopoietic stem cell or myeloid antigen. Early sequencing efforts in childhood ETP ALL uncovered a mutational landscape that is distinct from non-ETP disease. Notably, pediatric ETP ALL was found to be enriched for activating mutations in genes regulating *RAS* and cytokine receptor signaling and inactivating mutations in genes related to hematopoietic development and epigenetic modification of histones [44]. Efforts aimed at describing the genomic composition of adult ETP ALL have yielded similar patterns, and mutations in genes traditionally associated with myeloid malignancies, such as *DNTM3A* and *RUNX1*, were particularly remarkable [45, 46].

While children with ETP ALL were initially thought to have poor outcomes secondary to chemotherapy resistance [47], further analyses have concluded that the high-risk biological features could be neutralized by timely MRD-directed salvage therapies [48]. An influential 2017 study by Bond and colleagues examined similar factors in a large adult cohort of T-ALL patients ($n=213$), 47 of whom had ETP ALL. As seen in the pediatric patient population, adults with the ETP phenotype had markedly higher resistance to corticosteroid and chemotherapy regimens, and tended to demonstrate higher rates of bone marrow MRD positivity; excitingly, these patients demonstrated similar overall survival rates compared with non-ETP patients in the context of MRD guided treatment strategies (5-year survival of 59.6% for ETP, 66.5% for non-ETP, $P=0.33$) [49]. Based on multivariate analysis, the authors concluded that despite a more resistant disease biology, the use of NGS-guided risk stratification, with prompt therapeutic intensification, and the decision to proceed to allograft in first complete remission was able to markedly enhance survival in the ETP group.

One final example regarding the way in which NGS may enhance our understanding of disease biology pertains to the presents of *PHF6* in T-ALL. *PHF6* is an X-linked tumor suppressor gene known to be part of the mutational fingerprint of T-ALL. *PHF6* mutations have been shown to occur in 16% of pediatric and 38% of adult primary T-ALL samples [50]. Notably, such mutations are also present in 25% of ETP-ALL cases. If inactivated by such a mutation, *PHF6* has been shown to enhance hematopoietic stem cell self-renewal and to propagate transcriptional networks associated with leukemic cell stem-cell activation. This facilitates hematopoietic stem cell reactivation/proliferation for prolonged periods after chemotherapy [51]. Additionally, some cases of *PHF6*-mutant T-ALL may share a common cell of origin with seemingly unrelated secondary malignancies, such as histiocytic neoplasms. We have previously described NGS-guided therapy selection in a patient with a rare histiocytic sarcoma which presented after stem cell transplant for T-ALL; this malignancy harbored a mutational profile suggesting a shared neoplastic cell of origin. Using sequencing to identify mutational composition and tumor mutational burden allowed for a deeper understanding of disease modeling and hypotheses surrounding the PHF6-mutant driven divergence of the malignant T-cell clone from a bipotential progenitor cell [52].

Germline Predisposition to ALL

While thorough efforts have been focused on the somatic mutational landscape of ALL, germline variants predisposing to the development of ALL and other hematopoietic malignancies have gained increased recognition in recent years. For decades, blood cancers have been noted to cluster in families, but only recently have the molecular drivers of hereditary hematopoietic malignancies (HHM(s)) begun to be elucidated [53, 54]. Although the incidence of pathogenic or likely pathogenic germline variants in pediatric oncology cohorts is about 8.5% [55], it is a misconception that cancer predisposition syndromes only manifest in childhood. For example, germline variants in DDX41, which likely represent the most common HHM and account for at least 1% of all myeloid malignancies, have been described almost exclusively in adulthood [56, 57]. At this time, the impact of HHM-associated inheritance and phenotypic manifestations is better studied for myeloid than lymphoid malignancies, and a provisional entry for "Myeloid Neoplasms with Germline Predisposition" was included in the 2016 World Health Organization classification revision [5]. However, the contribution of heritable variants and the future development of lymphoid disorders remains an area of active investigations. Table 2 highlights several important HHMs associated with predisposition to ALL and two high yield syndromes are discussed below.

The *PAX5* gene codes for a DNA-transcription factor with a critical role in B-cell differentiation and somatically

Table 2 Select germline predisposition syndromes in acute lymphoblastic leukemia. A selection of genes associated with inherited predisposition to acute lymphoblastic leukemia are described by their inheritance, cellular function, and phenotypes. Review articles are favored in references, but original investigations are also cited. Abbreviations: AD, autosomal dominant; AR, autosomal recessive; T-ALL, T-cell acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndromes

Gene	Cellular function	Inheritance pattern	Hematologic features; other clinical phenotype	References
ATM	DNA repair	AD	T-ALL, CLL, lymphomas; prostate and breast cancers	[75, 76]
BLM	DNA repair	AR	B-ALL, MDS, AML, lymphomas; short stature, immunodeficiency, photoreactive rash	[77]
ETV6	DNA transcription	AD	B-ALL, multiple myeloma, platelet dysfunction, thrombocytopenia	[78]
IKZF1	DNA transcription	AD	B-ALL	[79]
NBN	DNA repair	AR	T-ALL, non-Hodgkin lymphoma; short stature, microcephaly, facial dysmorphia, immunodeficiency	[80]
PAX5	DNA transcription	AD	B-ALL	[61, 79]
PTPN11	Cellular proliferation	AD	B-ALL, juvenile myelomonocytic leukemia; dysmorphic facies, short stature, cryptorchidism, cardiac anomalies, coagulopathy	[81]
RECQL4	DNA repair	AR	T-ALL, lymphomas; osteosarcoma, premature aging	[82]
RUNX1	DNA transcription	AD	B-ALL, T-ALL, multiple myeloma; platelet dysfunction, thrombocytopenia	[83]
TP53	DNA transcription	AD	B-ALL, T-ALL, MDS, AML, lymphomas; sarcomas, Wilms tumor, multiple solid tumors	[83]

acquired variants, in the form of pathogenic single nucleotide mutations, copy number alterations, or chromosomal rearrangements are common. Disruption in native PAX5 function has been shown to arrest B-cell development [58, 59]. The 2013 report of two unrelated families with autosomal dominantly inherited pre-B ALL established *PAX5* as a new HHM syndrome. In both kindreds, germline PAX5 variants segregated with chromosome 9p loss, leading to a hemizygous variant allele [60]. More recently, Duployez and colleagues described a novel R38H *PAX5* germline variant in a family devastated by the development of B-cell precursor ALL in three children, ages 11, 17, and 25 and a post-transplant donor-cell derived relapse involving two of the siblings [61]. From reports thus far, it does not appear that HHMs driven by germline *PAX5* variants have an antecedent hematologic phenotype, such as cytopenias, before the development of B-ALL. Parental carriers in the affected families were asymptomatic, and unlike other HHMs, immunodeficiency was not a feature of either the carrier or diseased state. Therefore, it is reasonable to postulate that the frequency of cases related to germline mutant PAX-5 driven ALL might be an underestimate, and the syndrome should be considered prior matched related donor workup for B-ALL. However, given the lack of clinical features before the onset of ALL and the recognition that heterogeneity exists within commercially available tests for familial hematopoietic malignancies [62], diagnosis remains challenging.

TP53 is among the most commonly mutated genes in human cancers, and pathogenic germline *TP53* variants are diagnostic of the Li-Fraumeni syndrome, which results in an enhanced risk for development of multiple tumor types, most prominently sarcomas, but also adrenocortical carcinomas, breast cancer, central nervous system cancers, and hematopoietic malignancies [63, 64]. Within the hematologic landscape, germline *TP53* variants are most prominently associated with low hypodiploid ALL. In a series of 140 hypodiploid ALL patients, *TP53* mutations were highly prevalent and in one pediatric cohort, 43.3% of variants were of germline origin [65]. Hypodiploid ALL is generally subclassified by patterns of chromosomal loss, with near haploid disease harboring 24–31 chromosome and low hypodiploid harboring 32–39. Additionally, each of these two subtypes are enriched for two distinct mutational patterns, with *RAS* pathway mutations being common in near-haploid ALL and mutations in *IKZF2* and *IKZF3* in low hypodiploid ALL [66, 67]. Large surveys of pediatric ALL have demonstrated germline TP53 variants in pediatric hypodiploid ALL (estimated to contribute to about 2% of all cases in a series of 3801 children) [68], but previously unrecognized germline variants can also be found in adult oncology patients. One study of breast cancer survivors who developed therapy-related leukemias found that 21% of carried deleterious germline variants. Of the 47 subjects diagnosed with therapy-related leukemia with specimens available for analysis, *BRCA1/2* lesions were the most common (10% of cases), while *TP53* variants comprised an additional 6% of cases. Because many of the genes noted to be mutated in therapy-related leukemias function to maintain the DNA damage response, future work will likely reframe our understanding of such events to a model whereby individuals harboring germline variants in critical genes are particularly vulnerable to the

genotoxic stress imparted by chemotherapy or radiation for primary tumors [69, 70].

Conclusions

With the more widespread adoption of molecular profiling for patients with all types of cancer, individuals with ALL are likely to continue to derive benefit from improved appreciation for the molecular drivers of disease, moreover, widespread adoption of uniform and sensitive techniques for assessment of MRD are likely to improve the choice of treatment for patients and drive escalation or de-escalation of therapeutic intensity in an increasingly data driven manner. Information derived from NGS mutational assessment both at the time of initial diagnosis, as well as at critical follow-up time points has already transformed the myeloid malignancy space and is poised to augment diagnosis, risk stratification, and relapse prevention in the future. Currently, the use of NGS in ALL most commonly takes the form of targeted panels that are employed upfront at academic centers for patients presenting with hematologic malignancies. We anticipate that further study of NGS data will help refine and clarify molecular subsets of ALL to augment traditional disease classification and risk assessment driven by cytogenetics and clinical features. NGS-based MRD for lymphoid malignancies has already been broadly adopted in the context of pediatric ALL, and also holds promise to enhance or replace flow cytometry practices as it requires less operational cost and expertise and is able to automated for high throughput workflow. Blinatumomab is currently approved as an MRD-directed ALL therapy, but detection of residual disease at this time is generally achieved by single gene PCR or flow-cytometry based methods. In the future, NGS-driven MRD assessment may integrate sequence detection for the immunoglobulin or T-cell receptor of the malignant clone with detection of additional mutational signatures to enhance molecular risk stratification. Lastly, we anticipate significant future opportunities for NGS to help widen the spectrum of genes involved in hereditary hematopoietic malignancies and the molecular mechanisms underlying their pathogenesis.

Author Contribution G.W.R. and E.A.G conceived and designed the article. A.A., A.R.H, and G.W.R. performed the primary literature review. All authors contributed to the writing of the manuscript. All authors approve of the final manuscript and are accountable for the work.

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Code Availability Not applicable.

Declarations

Conflict of Interest Dr. Griffiths has received honoraria/advisory board payments from Alexion Pharmaceuticals, Celgene/BMS, AbbVie, and Novartis. EAG has also received institutional research funding from Genentech Inc., Appelis pharmaceuticals, Celldex Therapeutics, and Celgene/BMS. The other authors declare no conflicts of interest/competing interests.

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