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INVITED REVIEW

Pediatric leukemia: Moving toward more accurate models

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Leukemia is a complex genetic disease caused by errors in differentiation, growth, and apoptosis of hematopoietic cells in either lymphoid or myeloid lineages. Large-scale genomic characterization of thousands of leukemia patients has produced a tremendous amount of data that have enabled a better understanding of the differences between adult and pediatric patients. For instance, although phenotypically similar, pediatric and adult myeloid leukemia patients differ in their mutational profiles, typically involving either chromosomal translocations or recurrent single–base-pair mutations, respectively. To elucidate the molecular mechanisms underlying the biology of this cancer, continual efforts have been made to develop more contextually and biologically relevant experimental models. Leukemic cell lines, for example, provide an inexpensive and tractable model but often fail to recapitulate critical aspects of tumor biology. Likewise, murine leukemia models of leukemia have been highly informative but also do not entirely reproduce the human disease. More recent advances in the development of patient-derived xenografts (PDXs) or human models of leukemias are poised to provide a more comprehensive, and biologically relevant, approach to directly assess the impact of the *in vivo* environment on human samples. In this review, the advantages and limitations of the various current models used to functionally define the genetic requirements of leukemogenesis are discussed.

Leukemia is a cancer of the blood, originating from hematopoietic stem and progenitor cells that lose their capacity for proper self-renewal, differentiation and apoptosis. While typically diagnosed in older adults (median age of ~68 [1–4]) this complex genetic disease still remains one of the most common cancer during childhood, representing almost one third of all cancer diagnoses in children under the age of 15 [5–7]. Large-scale genomics studies of adult Acute Myeloid Leukemia (AML) patient cohorts have shown that it is a genetically heterogeneous disease, with a complex mutational landscape that has complicated efforts to develop broadly applicable targeted therapies [8]. Evidence suggests that in adult patients, the gradual acquisition of random mutations, some of which have oncogenic activity, over several decades can convert a normal hematopoietic stem cell into an unregulated leukemic blast [9]. Recently, similar studies of large cohorts of pediatric acute myeloid leukemia patients have confirmed past observations that, in contrast to the gradual accumulation of mutations seen in adult AML, in younger AML patients, chromosomal translocations are most often responsible for the development of the disease [10], in contrast to pediatric

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vs adult ALL [11]. Even mutations that have been shown to be recurrent in both adult and pediatric AML patients occur at highly divergent frequencies [10]. Taken together, current evidence suggests that despite the common phenotypes of pediatric and adult AML, they largely represent distinct genetic diseases with chromosomal rearrangements likely representing the initiating (or driver) event in a large fraction of childhood leukemia development.

Patient outcomes for childhood leukemia have significantly improved in the last 40 years [12], largely through improved treatment modulation; however, they still remain lower in AML than in most other pediatric cancers [13]. In addition, patient outcomes are highly dependent on the specific subgroup of leukemia involved [10], suggesting that distinct mutational profiles, and therefore therapeutic targets, exist. As noted previously, individual genetic heterogeneity is observed in all leukemia subgroups, confounding efforts to define the essential genetic elements required for leukemias with specific translocations or other genetic driver events. It is therefore critical to develop fully representative models of each leukemia type, such that the genetic background noise that comes with studying individual patient samples can be effectively removed.

Among the first experimental tools developed to model the biology underlying the disease were cell lines established from the *in vitro* culture of primary patient samples. After their initial establishment, leukemic cell lines represented a relatively simple and cost-effective way to study the disease, and have formed the backbone of much of the experimental work performed to date. More recently, large-scale studies of leukemic cell lines have also provided deeper insight into drug sensitivities [14] and genetic networks that are active within cell lines [15,16]. Despite their utility, all cell lines have undergone selection for their ability to grow in an *in vitro* environment, typically without growth factors or other signals primary leukemias typically receive in the bone marrow niche. As a result, the gene expression patterns and underlying molecular biology often are not representative of the disease seen in patients.

In parallel efforts, concerted studies aimed at better characterizing mouse hematopoietic stem/progenitor cells (HSPCs) have subsequently allowed the development of murine leukemic models to explore disease mechanisms *in vivo* [17–19]. Such models represent an extremely powerful tool, not only for defining the genetic dependencies of leukemias, but also for providing an experimental system in which to evaluate therapeutic approaches. Although a range of leukemic models have been developed, many of these involve the use of the potently oncogenic gene fusions typically seen in pediatric leukemias [20–23]. While potentially closer to the human disease than cell lines because of the lack of selection for *in vitro* growth, differences in genome structure, life span, and transformability of mouse cells mean that some of these models still do not fully recapitulate the human disease. Clearly, the ability to generate human models of pediatric leukemia would represent an optimal experimental system; however, the practical development of such models has been hampered by technical challenges in isolating and transforming the correct HSPC population and identifying the correctly optimized culture conditions and the availability of suitable mouse strains. Once again, these road blocks have been overcome by transducing HSPCs with potently oncogenic fusion genes such as KMT2A–MLLT3 (MLL–AF9), allowing engineered human leukemias to be produced [24].

In this review, we discuss the various benefits and drawbacks of the various different model systems available to study pediatric leukemias, including cell lines and human models. Additionally, we present descriptions of the current public patient-derived xenograft (PDX) resources available, and describe their impact on the development of novel therapeutics.

Mining patient cohorts to understand the disease

In the past decade, rapid advances in DNA sequencing technology have fueled the field of cancer genomics, enabling the comprehensive sequencing of large cohorts of patient tumors and matched normal tissue [25,26]. The vast data sets created through these efforts has opened a powerful window to understanding the genetics of cancer biology including recurrent mutations, gene expression, and chromatin accessibility [27]. Comparisons of the mutational burden in different cancer types have shown that acute myeloid leukemias have significantly fewer somatic mutations present relative to other cancers [28], with pediatric AML having even lower levels than adult AMLs [10]. Despite this fact, high levels of genetic heterogeneity exist between all cancer patients, including pediatric leukemia patient samples. Such heterogeneity includes not only recurrent somatic mutations, but also the 200–300 [29,30] loss-of-function variants and thousands of structural variations [31] estimated to be present in each human genome. Clearly this genetic variation has the potential to influence the development of leukemia, though likely through complex interactions of other variants or somatic mutations, which may be patient specific. Although studies of patient cohorts provide an important "average view" of a cancer, a comprehensive understanding of the contribution of genetic drivers in individual patients (i.e., how all of their somatic and germline variants contribute to the leukemia) is often missing.

Several large-scale genomics studies, focusing on pediatric cancers [32] or specifically on leukemias [10,33–37], have been published in which the frequency of recurrent translocations is typically high.

One landmark project, the TARGET (Therapeutically Applicable Research to Generate Effective Treatments) program, is managed by the National Cancer Institute, the Children's Oncology group, St. Jude Children's Hospital, and others and has the goal of using a multi-omics approach to comprehensively define all the molecular changes that are specifically implicated in the initiation and progression of childhood cancer. The data from this project, much of it publicly available, have been of significant value in investigating common disease mechanisms and identifying novel therapeutic strategies for hard-to-treat pediatric cancers. For instance, a pan-cancer genome analysis of 1,699 pediatric leukemias reported 142 potential driver genes, of which only 45% were also found in adult patients, highlighting the need for the development of distinct treatment strategies for young patients [38]. Another more specifically focused study characterized nearly 1,000 young and adult pediatric patients and provided an outstanding molecular landscape of AML [10]. Supporting previous evidence on the differences between adult and pediatric leukemia, the authors provided clear evidence for age-specific differences in the spectrum of mutations and genetic variations present. Mutations that are typically found at high frequency in adult patients (e.g., FLT3, NPM1, IDH1, and IDH2) are rare in pediatric patients, whereas the frequency of mutations in specific signaling pathways (e.g., KRAS, NRAS, KIT, WT1) is significantly higher [10]. Moreover, the incidence of recurrent translocations in AML also follows an age-specific pattern, a fact likely linked to the early development of leukemia. Of all gene fusions, those involving the KMT2A (mixed lineage leukemia [MLL]) gene are the most common translocations seen in infants and older children [10,39]. With respect to recurrent fusions in pediatric B-cell (B-ALL) and T-cell (T-ALL) acute lymphoblastic leukemia, many of these have been identified and functionally characterized as well. For instance, the ETV6–RUNX1 (TEL/AML1) fusion resulting from the often cryptic t (12;21) translocation is one of the most common fusions and is found in 25% of pediatric ALL patients [40], where its occurrence during pregnancy may provide a "first hit" for the leukemia [41]. The well-documented Philadelphia chromosome resulting from the fusion between ABL1 and BCR (t(9;22)(q34;q11)), which is often observed in adult B-ALL, is much less frequent in pediatric cases of B-ALL (~25% vs. 4% of cases [42]). Interestingly however, in Philadelphia chromosome-like B-ALL (representing ~20% of all pediatric patients [43]), fusions of the CRLF2 gene were seen in 43% of patients, whereas those without CRLF2 fusions were frequently characterized by JAK2/EPOR fusions (~8% of cases) [43]. The most common gene fusion in pediatric T-ALL involves the STIL–TAL1 genes leading to an overexpression of TAL1 (along with frequent overexpression of SLC17A9) not seen to the same extent in adult T-ALL [44], while a diverse collection of other recurrent fusions have been identified [45]

Another large-scale genomics effort, the Pediatric Cancer Genome Project (PCPG) cooperatively developed in 2010 by St. Jude Children's Research Hospital and Washington University analyzed the genomes of childhood cancer patients and identified somatic mutations that drive cancer [46]. With roughly 5,000 patient samples from more than a dozen cancer types, including more than 2,300 leukemic samples, these data represent another highly informative source for pediatric cancers, complementing others available through the US National Human Genome Research Institute (NHGRI), National Cancer Institute (NCI), The Cancer Genome Atlas (TCGA), the International Cancer Genome Consortium (ICGC), and the TARGET project. Importantly, the findings from all of these studies have emphasized the challenges involved in analyzing the complex genetic data, where age-dependent differences complicate comparisons between adult and pediatric patients. For example, although the frequency of somatic mutation is lower in pediatric AML patients than in adult patients, there may be a greater role for germline variants that predispose pediatric patients to AML. Such predisposition variants have been identified in pediatric ALL [47,48], AML [49], and multiple myeloma [50] patients and are estimated to occur in ~10% of all pediatric cancers [51]. Given that no comprehensive list of such variants exists for pediatric AML, and that the 10% frequency reported may be an underestimate [52], comparing the genetics of pediatric AML and adult AML through recurrently mutated genes is problematic. This, in turn, has highlighted the critical need for better models of the disease to be able to functionally study the role of specific mutations in the disease and to develop genotype-specific treatments.

Using cell lines as model systems for pediatric leukemias

Long before the advent of recombinant DNA technologies, immortal cell lines derived from patient tumors had already been developed as a model to try to better understand the biology of cancer. Over the years, hundreds of cell lines have been established from a range of different tumor types, and like current cancer patient samples, NGS approaches have recently been applied to provide a detailed molecular characterization of large sets of these cells [14,53–55]. With respect to pediatric leukemias, a number of cell lines have been established from patients [56], many of which contain recurrent gene fusions seen in many patient leukemias. At the same time, this effort has been complicated by

the fact that primary leukemic cells will rapidly differentiate or die when placed into *in vitro* culture. Even those cells that do not immediately die must often be cultured for months [57] before a proliferating clone emerges, resulting in a highly inefficient process for generating cell lines. Once established, such cell lines have, however, provided a useful, inexpensive, and simple model to study defects in cell differentiation and self-renewal leading to the development of a specific leukemia. They also represent a powerful system for high-throughput chemical or genetic screens and for testing the impact of specific mutations. Moreover, the large number of different cell lines representing various tissues provides the possibility of not only assessing the consistency and reproducibility of results but also examining the tissue-specific aspects of experiments.

Despite their immense value, virtually all cell lines are in fact far removed, biologically speaking, from the actual human disease for a number of reasons. For instance, by virtue of how they are established, all tumor cells are selected for the "unnatural" characteristic of being able to grow *in vitro*. For leukemic cells, basic *in vitro* culture conditions typically lead to rapid cell differentiation and death without the addition of additional growth factors or stromal support cells [58]. As a result, these stringent growth conditions can rapidly select for any subclones harboring mutations that allow them to survive, even if these would have no advantage *in vivo*. Moreover, the constant passage of cells also has the potential to apply selective pressure for faster growth, leading to the accumulation of additional mutations or genomic instability not seen in the primary disease, and is often reflected in their highly variable karyotypes. There is clear evidence of the dramatic effects of this process in other cancer cell lines [59], and although evidence for similar genetic heterogeneity has not yet been described for AML cell lines, an immortal and continually growing cell line with finite DNA replication fidelity must inevitably acquire novel mutations. In addition, the karyotypic abnormalities seen in large numbers of AML cell lines [60] likely also reflects an inherent level of genomic instability that can affect the phenotype of the cell lines. For instance, analysis of the gene expression patterns of ~200 hematopoietic cell lines reveals that their morphological classification does not always correlate well with their clustering based on expression patterns of different leukemia subtypes (Figure 1). As to whether any specific AML cell line may be a more representative model of pediatric AML, it is difficult to say, and this likely depends on the presence of specific mutations or biological activities that are being examined.

Attempts to overcome these inherent limitations of cell lines have largely focused on using large numbers of cells to observe the general trends despite the genetic noise present. Several large-scale studies, using hundreds of cell lines, have been performed to test tumor response to drugs to identify novel therapeutic targets [14,53]. While similar caveats remain (e.g., that drugs showing therapeutic effects *in vitro* may not have the same consequences on cancerous cells because of differences in the *in vivo* microenvironment [61]), these data can still be informative for understanding the relevance of specific biological pathways. Human AML cells lines bearing gene fusions commonly seen in pediatric leukemias have been the focus of a number of high-throughput studies aimed at establishing a catalog of genetic vulnerabilities. Approaches using both shRNA- [62–64] and CRISPR-Cas9–based [65,66] screening approaches have provided useful insight into the biology of pediatric leukemia despite clear differences caused by cell line–specific genetic heterogeneity (e.g. both differences between cell lines and subclonal heterogeneity with cell lines). Taken together, even though cell lines provide a useful resource to study the genetics of leukemias, they still present potential limitations for specific biological questions, and studies using individual cell lines may not provide accurate data for attempts to recapitulate the disease *in vivo*.

Murine models of pediatric AML

Given the inherent limitations of cell line models of pediatric leukemia, the generation of novel models starting from primary cells has been of great interest. Such models have in turn been dependent on parallel technical advances in the ability to isolate and characterize murine progenitor cells that are competent to be transformed. Initial efforts to transform murine cells involved the prototypical BCR–ABL fusion seen in adult chronic myelogenous leukemia (CML) and used retroviral transduction of murine bone marrow (BM) cells [67,68]. These models illustrated that it was feasible to introduce specific oncogenes into murine cells to transform the cells into a state that can mimic the human disease. Subsequent models established through the homologous recombination of common pediatric gene fusions such as the KMT2A gene were performed using murine embryonic stem (ES) cells and indicated that, phenotypically, the transformed cells resembled human AML [23]. The high frequency of KMT2A translocations in pediatric leukemias [69], its astonishing range of fusion partners [70], and the success of initial studies all serve to explain the numerous subsequent efforts to study similar models with KMT2A fusions with ENL and AF4, among others. Interestingly, a number of oncogenic fusions seen in patient leukemias are insufficient by themselves to transform murine HSPCs (e.g. ETV6–RUNX1) or do so only after long latency periods (e.g. MLL–AF4). This observation suggests that in some cases, the ability to

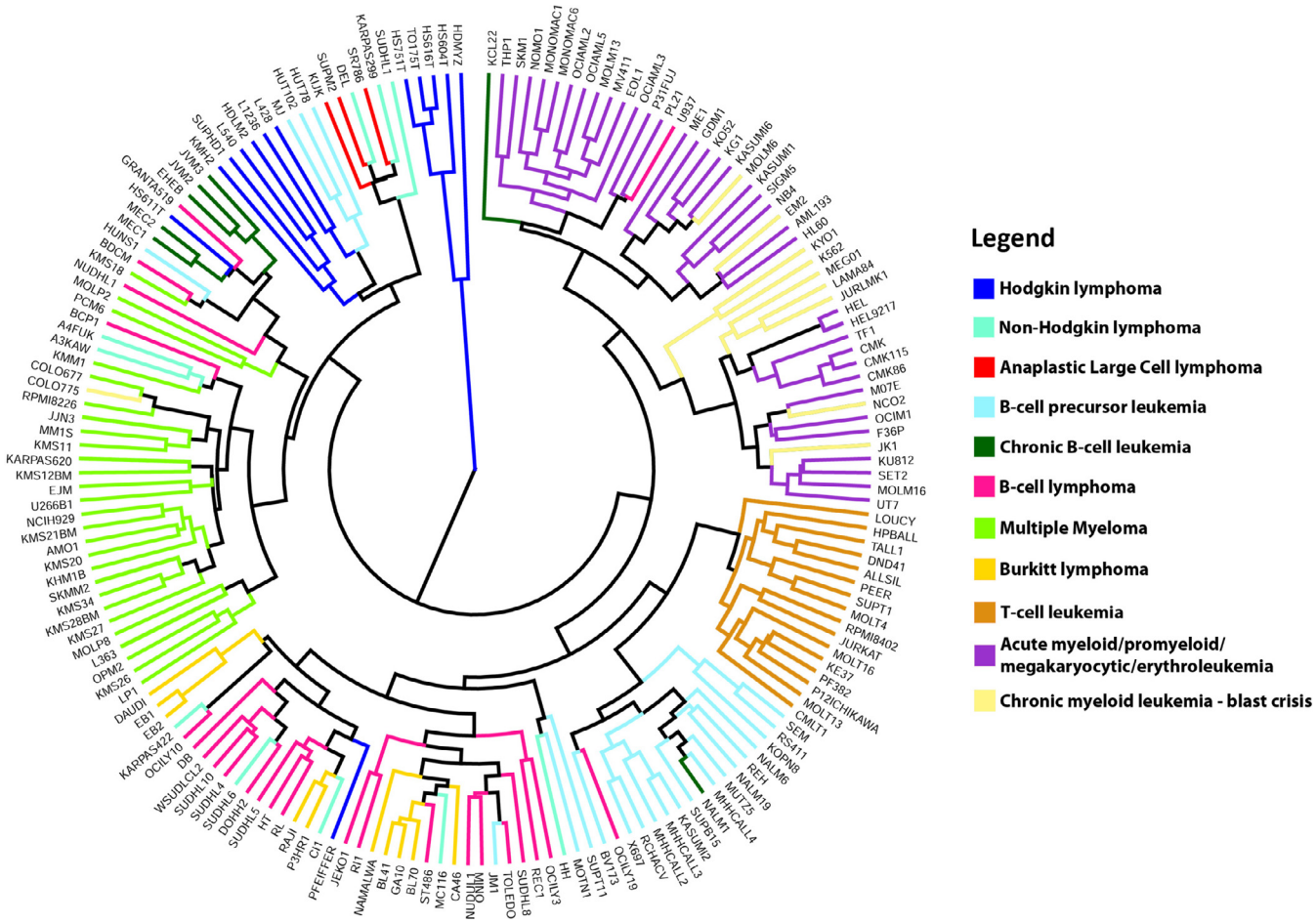

Figure 1. Hierarchical clustering of hematopoietic cell lines. Published gene expression data from the Cancer Cell Line Encyclopedia analysis of (~200) established hematopoietic cell lines was used to perform hierarchical clustering based on the expression levels of the 1,000 most variable genes across all cell lines. Cell type annotation derived from published descriptions as well as information from commercial cell repositories was used to color the dendrogram branches for the cell types noted in the legend.

accurately recapitulate certain human leukemias will likely require a better understanding and quantification of the contribution of typically heterogenic mutations seen in patient samples or isolation of relevant cell of origin for distinct leukemia subtypes.

The cell of origin used for the model leukemias that have been developed is also an important consideration for the accurate duplication of disease phenotypes across species. Although some earlier models using genetically manipulated embryonic stem cells from transgenic mice have been found to generate leukemias, most fusions in pediatric leukemias are typically tumor restricted along with recurrent somatic mutations. The continual refinement of cell surface markers for murine HSPCs has meant that many of the later models have specifically targeted these cells for transformation. Despite this more precise targeting, even relatively pure HSPC populations remain heterogeneous with a range of differentiation potential [71] whose impact on leukemia development (e.g. lineage phenotype) remains undefined. In the case of particularly potent oncogenes such as KMT2A fusions, the cell of origin (or even the promoter driving the fusion [72]) may have a negligible impact on leukemia development. This may not be the case, however, for other oncogenic fusions that have been used to model pediatric leukemias, including AML1–ETO [73], E2A–PBX1 [74], and, more recently, models looking at higher risk groups such as acute megakaryoblastic leukemia (AMKL) [20].

The creation of murine models of pediatric leukemia has been particular informative for performing functional studies to define the essential genetic requirements for leukemia development from normal progenitor cells. The ability to perform these experiments iteratively, under a range of different conditions of genetic perturbation, provides significant flexibility to investigate specific genes. Although similar experiments can be performed in individual leukemia cell lines, in this case the leukemia is already maintained by a specific collection of genetic lesions that may or may not be relevant for a given patient subgroup, limiting the scope of experiments. At the same time,

because mouse models are typically designed to address the role of specific proteins or mutations, testing complex combinations of the 4–10 driver mutations found in adult AMLs [75] is not feasible (Table 1).

Human models of pediatric leukemia

One of the most significant constraints on the functional study of pediatric leukemias, aside from their rarity, is the fact that there is typically very little material available for studies. To overcome this challenge, immunocompromised (NOD-SCID, NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ)) mice have been used to expand these limited tumor cells through xenografting of primary patient cells. In the case of both ALL [76,77] and AML [78], a leukemia similar to the patient sample can be expanded. These PDXs have differing success rates (60–75% [79–81]) based on the permissibility of the recipient mice [82,83], the specific leukemia sample used, and the presence of cytokines and growth factors that can influence engraftment success [84]. Such PDX models present multiple unique advantages, both in fundamental research and in a clinical setting, including the ability to preserve stromal components in the context of orthotopic engraftment and the ability to be used in *in vivo* functional studies including screening of drugs capable of targeting the patient's tumor [83,85,86]. Given the potential value of PDX samples, it is not surprising that numerous academic and commercial repositories have been established, including ProXe [87], HuBase (CrownBio), EurOPDX [88], and PROPEL (St Jude Children's hospital), along with meta-search services such as PDXFinder [89] (covering almost 2,000 available PDXs in eight repositories). These repositories, some of which include pediatric leukemia PDXs, allow researchers to easily obtain viable tumor samples, many of which have also undergone significant initial molecular or genomic characterization. These collections of PDX tumors, searchable by tumor type, specific mutations present, and drug dosing information, give researchers unprecedented power in selecting relevant tumor samples for functional tests.

Although PDXs represent a powerful resource, questions remain as to the extent to which selective conditions within the mouse alter the phenotype of the original tumor and how stable the tumor genotype remains. For example, recent large-scale studies have reported a high frequency of copy number alternations within four cell passages, along with expansion of minor clones within the initial tumor [90]. And although in AML PDXs, a majority of driver mutations remain concordant with the primary tumor (even when serial transplantations are performed [81]), some somatic variants present at a low frequency in the primary tumors (e.g., variant allele frequency: 10–30%) exhibit large changes (>two fold) in PDXs [80]. Despite these observations, PDXs clearly provide a complementary approach to functional studies of patient tumors, and it remains to be seen how such alterations relate to similar changes seen during tumor evolution, driven by the selective pressure of patient treatment.

Patient-derived xenograft models such as those described above have been useful in removing some experimental constraints; however, the use of xenografts still presents a limitation with respect to accurately reproducing the human bone marrow niche and immune environment, which can play a critical role in tumor development and progression [91–93]. Moreover, although the engraftment of human peripheral blood mononuclear cells (PBMCs) or reactive T cells can provide some immune activity against the human tumor, the lack of human antigen presenting cells (APCs) and development of host-versus-graft responses limit the value of this approach. This limitation has been addressed by the development of a humanized mouse model, where sublethally irradiated immunodeficient mice received transplanted fetal thymic tissue (FTHY) and CD34^{+} fetal liver cells (FLCs) [52]. Such humanized mice, when challenged by KMT2A–MLLT3-bearing leukemia cells, exhibited an immune response similar to that seen in patients while eliciting a more representative immunological response to the disease [53].

Given the species differences between murine and human cells and the potential genetic differences induced through the generation of human PDXs, the ability to generate models of pediatric leukemia starting from normal human HSPCs would represent an attractive solution. Interestingly, similar genetic approaches using human hematopoietic progenitor cells have been slow to develop because of the technical challenges involved in achieving the levels of transformation seen in mouse progenitor cells. Although the differences responsible have not been completely defined, studies looking at different HSPC populations indicate that HSPC transformational competency decreases in cells with age (cord blood vs. bone marrow) [94] and location (bone marrow vs. peripheral blood) [94]. This observation also agrees with age-dependent changes in the competence of specific progenitor cells to be transformed in the case of pediatric leukemias that are extremely rare in adults (e.g. AMKL [95]).

One of the first successful demonstrations of a human model leukemia was performed using potent KMT2A fusions such as MLLT3 [24]. The derived leukemias phenotypically resembled patient samples and were transplantable but, unlike primary AMLs, were also able to be maintained in *in vitro* culture for extended periods. Subsequent detailed genomics studies on similar single donor-derived leukemias also confirmed the generally held hypothesis that the fusion gene itself was sufficient to generate the leukemia and that no recurrent secondary mutations were required [96]. Other human leukemia model fusions have not only illustrated the importance of the fusion partner in

Table 1. Human and mouse models of leukemia

Species	Year	Driver used	Phenotype of model	Notes	Reference
Mouse	2018	GATA2−HOXA9, MN1−FLI, NIPBLHOXB, (CBFA2T3−GLIS2)	AMKL	CBFA2T3−GLIS2 fusion alone is insufficient to generate leukemia	[20]
	2017	ETV6−RUNX1	B-Cell precursor ALL	Loss of function in KDM family genes potentially relevant for disease penetrance	[107]
	2015	E2A−PBX1	B-Cell precursor ALL	Pre−BCR signaling/JAK kinases are potential therapeutic targets	[74]
	2013	MLL−AF6	AML	Selective sensitivity to Dot1l inhibitor (EPZ0004777)	[108]
	2009	ETV6−RUNX1	Increased HSPC frequency but no leukemia	Chemical mutagenesis required for leukemia development	[109]
	2009	OTT−MAL	AMKL	Notch signaling (RBPJ) and cytokine (MPL) signaling are required for AMKL generation	[110]
	2008	MLL−AF4	B−ALL/AML	H3K79 methylation patterns correlate with gene expression patterns and are conserved between human and mouse samples	[111]
	2006	MLL−AF4	B−ALL	Expression of MLL−AF4 restricted to lymphoid cells, drives B−ALL development	[72]
	2006	MLL−AF4	B−ALL/AML	Both MLL−AF4/MLL−AF9 transform BM cells, but MLL−AF9 has a shorter latency and favors AML vs. BALL/MPAL for MLL−AF4	[112]
	2005	AML1−ETO	AML	Demonstrated that AML1−ETO fusions collaborate with FLT3 internal tandem duplications to generate AML	[113]
	2003	MLL−ENL	AML	Rapid highly penetrant leukemias result from Cre−loxP-mediated reciprocal chromosomal translocations	[22]
	2003	NUP98−HOXD13	Myeloproliferation/AML	Full transformation of BM cells into AML was dependent on co−expression of MEIS1	[114]
	2002	ETV6−RUNX1	B−ALL/T−ALL	Development of leukemia is potentiated by loss of p16/p19	[109]
	2002	AML1−ETO	AML	Altered HSC compartment size but without leukemia of disseminated disease	[73]
	2001	NUP98−HOXA9	AML	Induces a polyclonal AML and defined a role for MEIS1 cofactor in accelerating the disease	[115]
	1996	MLL−AF9	AML	First mouse model of AML generated through Cre−loxP mediated fusion in ES cells	[23]
Human	2018	MLL−AF9	AML	CRISPR−based editing can be used in human hematopoietic stem and progenitor cells to generate leukemias	[116]
	2017	RBM15−MKL1	HPSCs with AMKL−like expression	Two HPSC cell lines expressing RBM15−MKL1 oncogenic fusion seen exhibit some gene expression patterns similar to that of AMKL	[101]
	2017	MLL−AF9	AML/B−ALL	RET highlighted as a therapeutic target and sequencing data showing the KMT2A−MLLT3 fusion alone is sufficient to generate leukemias	[96]
	2015	MLL−AF9/ENL	ALL/AML/MPAL	TALEN induced gene editing of the endogenous MLL gene in CD34 cells	[97]
	2014	NUP98−HOXD13	AML	Forced expression of MN1 is insufficient to generate leukemias in the absence of HOX gene fusion	[100]
	2012	MLL−AF4	Block in HSC commitment	Enforced expression of MLL−AF4 in hESCs impairs hematopoietic development and is insufficient to transform cells	[98]
	2012	MLL−AF9	AML	Intrinsic properties of the cell of origin affect the efficiency of transformation in human model leukemias	[94]
	2012	MLL−AF10	HSPCs with MLL−AF10 and activated K−ras develop AML	MLL−AF10 alone is insufficient to generate AML in humanized mice	[117]
	2011	MLL−AF4	HSPCs with increased proliferation and clonogenic potential	MLL−AF4 enhances proliferation but is insufficient to generate a leukemia in human cells	[118]
	2010	BCR−ABL	CML	BMI1 collaborates with BCR−ABL in leukemia development; lymphoid phenotype *in vivo* with myeloid/lymphoid cells established in culture	[119]
	2008	MLL−AF9	AML/ALL/MPAL	Phenotype of transformed CB cells is impacted by growth factors, microenvironment, and mouse strain used	[120]
	2007	MLL−AF9/ENL	AML/B−ALL	First human model of AML developed using CB cells	[24]

the leukemia [97,98], but have also clearly highlighted differences between murine and human models with the same oncogene [99], underlining the importance of human model systems. As with the murine models, many of the oncogenic fusions used in initial human models involved the KMT2A gene, which has strong oncogenic potential. Although other fusions have been tested in human models, such as the NUP98–HOXD13 fusion gene [100] for AML and the RBM15–MKL1 fusion gene [101] in the case of AMKL, these models have exhibited partial or weak leukemia penetrance or dependence on other genetic alterations to generate a leukemia. Given the fact that human HSPCs seem to be more resistant to transformation compared with their murine equivalent, it remains unclear how straightforward the development of other human models will be, even given the technical advances demonstrated through direct genome editing. Nevertheless, the existing models of pediatric leukemia indicate that with optimized constructs and culture conditions, it is possible to generate a human model of the disease that, importantly, closely recapitulates its behavior and phenotype.

With the development of both murine and human models of pediatric leukemia, there has been a great interest in using these models for functional studies to uncover the genetic underpinnings of the disease. Although space constraints preclude including an exhaustive list of these studies, a representative sample is presented in Table 2. Overall, these efforts have provided highly valuable insight into the molecular mechanisms involved in specific models of leukemia and have resulted in the advancement of several specific compounds to clinical trials [102–104]. At the same time, given the variety of screening approaches applied to the models (including siRNA and shRNA knockdown, or CRISPR-based screens), it is perhaps not surprising that the consistency of essential targets identified is relatively low. In fact, this diversity recapitulates the range of inhibitory responses seen in adult primary AML samples when challenged with a diverse collection of small molecules [105]. If the underlying heterogeneity of somatic and germline variants in AML patients does explain the diversity of response seen, it suggests that although models of pediatric leukemia will be useful for dissecting specific pathways, their ability to identify "universal" therapeutic targets may be inherently limited. Moreover, the model leukemias used for such functional studies are heavily biased toward the subset in which strong oncogenic drivers have been validated. As a result, their ability to uncover relevant therapeutic targets for the range of other pediatric subgroups (that are perhaps less frequent but have clinical outcomes that are just as poor) remains unclear. These challenges notwithstanding, the ability to reproducibly generate a range of experimentally tractable human model leukemias, from multiple HSPC

Table 2. Screens performed using human or mouse leukemia models

Species	Year	Format	Method	Phenotype of cells screened	Screen hits	Reference
Mouse	2018	*In vitro/in vivo*	CRISPR	AML	DCPS	[121]
	2017	*In vivo*	CRISPR	MLL–AF9, HOXA9, Meis1	B4galt1	[122]
	2015	*In vitro/in vivo*	shRNA	B–ALL	Phf6	[123]
	2014	*In vitro*	shRNA	MLL–AF9, HOXA9, Meis1	Jmjd1c	[124]
	2013	*In vivo*	Small molecule	MLL–AF9	Lovastatin	[125]
	2013	*In vivo*	shRNA	MLL–AF9	Itgb3	[126]
	2011	*In vitro/in vivo*	Small molecule	MLL–AF9/ENL	Brd3/4	[127]
	2011	*In vitro*	shRNA	MLL–AF9	Brd4	[128]
Human	2018	*In vitro*	shRNA	MLL–AF9	CHD4	[62]
	2017	*In vitro*	shRNA	MLL–AF9	ZEB2	[63]
	2017	*In vitro*	Small molecule	AML	Diverse	[105]
	2017	*In vitro/in vivo*	shRNA	AML	RET	[96]
	2017	*In vitro*	CRISPR	AML	PREX1	[65]
	2016	*In vitro*	CRISPR	Five leukemia cell lines	DOT1L, BCL2, Men1	[66]
	2015	*In vitro*	RNAi	AML	BNIPL1, ROCK1, RPS13, STK3, SNX27, WDHD1	[129]
	2012	*In vitro*	shRNA	AML	WEE1	[64]
	2012	*In vitro*	shRNA	AML	GSK–3α	[130]

sources that could potentially be genetically manipulated beforehand [106], will provide an essential resource that may provide more flexibility than the generation of PDXs in which the engraftment is variable.

Conclusions

Given the limits of either the static information from patient cohorts, or murine models that, in some cases, do not recapitulate all aspects of the human disease, the need to create new models that more closely reflect human cancer biology is clear. Such tools will be essential in understanding the stepwise requirements for leukemogenic transformation that will underpin the next generation of rationally designed, and genotype-specific, treatments. Many technical challenges remain, however, in identifying the genetic requirements of individual fusions to transform human progenitor cells to the correct phenotype. For this, the availability of both patient-derived xenograft models and engineered human model leukemias provides a range of biological tools to explore the functional dependencies. Whether PDX models or engineered leukemias are "superior" remains something of an open question, and so for the moment, it is most useful to view them simply as complementary systems, each well suited to solving different problems related to our incomplete understanding of leukemia biology and novel therapeutic avenues.

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