



Molecular therapeutic approaches for pediatric acute myeloid leukemia

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Approximately two-thirds of children with acute myeloid leukemia (AML) are cured with intensive multi-agent chemotherapy. However, refractory and relapsed AML remains a significant source of childhood cancer mortality, highlighting the need for new therapies. Further therapy intensification with traditional cytotoxic chemotherapy in pediatric AML is not feasible given the risks of both short-term and long-term organ dysfunction. Substantial emphasis has been placed upon the development of molecularly targeted therapeutic approaches for adults and children with high-risk subtypes of AML with the goal of improving remission induction and minimizing relapse. Several promising agents are currently in clinical testing or late preclinical development for AML, including monoclonal antibodies against leukemia cell surface proteins, kinase inhibitors, proteasome inhibitors, epigenetic agents, and chimeric antigen receptor engineered T cell immunotherapies. Many of these therapies have been specifically tested in children with relapsed/refractory AML in Phase 1 and 2 trials with a smaller number of new agents under Phase 3 evaluation for children with *de novo* AML. Although successful identification and implementation of new drugs for children with AML remain a formidable challenge, enthusiasm for novel molecular therapeutic approaches is great given the potential for significant clinical benefit for children who do not have other curative options.

Keywords: acute myeloid leukemia, clinical trial, demethylating agents, monoclonal antibodies, pediatric, precision medicine, tyrosine kinase inhibitors, targeted therapy

INTRODUCTION

Acute myeloid leukemia (AML) is a group of genetically heterogeneous diseases that account for approximately 20% of pediatric leukemias with approximately 800 newly diagnosed children and adolescents annually in the United States (U.S.) (1, 2). Current intensive cytotoxic chemotherapy regimens achieve long-term cure in only 60–70% of patients, however, and relapsed AML accounts for more than half of childhood leukemia-related deaths (3). Identification of specific genetic subgroups within AML and correlation with relapse-free and overall survival (RFS and OS, respectively) rates have allowed risk stratification of many patients and have decreased use of adjuvant hematopoietic stem cell transplantation (HSCT) for favorable risk group patients (1, 2). Nonetheless, chemotherapy regimens for AML have remained largely unchanged for the past four decades. Relapsed and chemotherapy-refractory AML is thus an area of unmet clinical need and presents opportunities for the development of novel targeted therapeutic approaches. New treatments for children with AML are clearly indicated to decrease relapse and improve cure rates.

Given the higher incidence of AML in adult patients and the limited success of conventional chemotherapy in maintaining long-term remission in this population, various new agents are under active investigation in early-phase clinical trials conducted within predominantly adult cooperative groups. Many of these novel agents aim to target genetic and molecular alterations

hypothesized to be involved in leukemogenesis and/or therapy resistance. However, progress in the development and successful implementation of new therapies has been limited by the underlying biologic heterogeneity of AML, the ability of older patients to tolerate intensive therapy, patients' associated medical co-morbidities, and rapid disease progression at time of relapse.

There is burgeoning evidence that the inherent biology of AML differs between children and adults, as suggested by the discordant incidences of leukemia-associated genetic alterations, patterns of epigenetic changes, and rates of remission induction (4–8). A comprehensive review of the biologic features and genomic landscape of pediatric AML will be published separately in this series. The relatively higher response rates of children to induction chemotherapy may be partially attributable to host factors, such as the generally superior ability of children to tolerate intensive multi-agent therapy, their lower prevalence of medical co-morbidities, and intensive supportive care measures. Nonetheless, primary chemorefractory disease and post-remission relapses remain significant sources of morbidity and mortality for children with AML, and re-induction attempts are frequently unsuccessful (2, 3). In addition, infectious complications and end-organ dysfunction sequelae of intensive chemotherapy occur frequently. Given these issues, investigation of new agents for high-risk childhood AML remains a high priority. Emphasis has been placed upon the development of molecularly targeted agents for childhood AML that will increase rates of successful remission induction,

decrease relapse by targeting leukemia-initiating cells, and minimize therapy-associated complications. Our review highlights the current landscape of novel molecular therapeutic approaches for childhood AML (excluding acute promyelocytic leukemia, APL) in clinical or late preclinical testing (Table 1).

ANTIBODY THERAPEUTICS

Approximately 80% of childhood AML cases express CD33, a glycosylated sialic acid-binding transmembrane receptor protein of the lectin family. High CD33 surface expression is associated with worse outcomes in children and adults with AML (9–12). The potent humanized anti-CD33 monoclonal antibody/calicheamicin conjugate gemtuzumab ozogamicin (GO) has been studied extensively in adults with AML, including APL. Three Phase 2 multi-center trials initially demonstrated second complete remission (CR2) rates of 30% and a favorable safety profile in adults with relapsed AML treated with GO monotherapy (13). These data resulted in accelerated approval of GO in 2000 by the U.S. Food and Drug Administration (FDA) for adults with AML, and other groups have reported similar findings (14, 15).

Gemtuzumab ozogamicin has been specifically studied in pediatric AML via the Children's Oncology Group (COG) and other consortia. Initial testing of GO monotherapy in the relapsed/refractory setting demonstrated similar pharmacokinetics to those in adult patients and morphologic remission rates of 28–54%, allowing many children to undergo successful subsequent HSCT (16–21). Studies from the St. Jude Children's Research Hospital (SJCRH) also demonstrated favorable molecular responses in GO-treated children with relapsed/refractory AML treated on the AML02 trial with 13 of 17 patients achieving flow cytometric minimal residual disease levels $<0.1\%$ (22). Pilot testing of GO with cytotoxic chemotherapy in children with relapsed/refractory AML also demonstrated the safety and tolerability of combination approaches, and complete remission (CR) was attained in nearly half of these high-risk patients in two independent studies (22, 23).

Based upon these data in the relapse setting, GO and chemotherapy combination regimens were subsequently evaluated in children with *de novo* AML. In the COG pilot trial AAML03P1, CR rates $>80\%$ were achieved in children treated with GO and chemotherapy in the induction and post-induction setting (24). In the NOPHO-AML 2004 study, post-consolidation addition of GO to chemotherapy was well-tolerated, but did not alter rates of relapse or OS (25). Most recently, children with *de novo* AML treated with chemotherapy and GO in induction and post-induction on the COG Phase 3 trial AAML0531 experienced decreased rates of relapse and increased event-free survival (EFS) in comparison to children treated with chemotherapy alone (26). Although induction mortality did not differ between treatment arms, a difference in cumulative treatment-related mortality (TRM) approached, but did not reach, statistical significance at rates of $8.6 \pm 2.5\%$ for GO/chemotherapy and $5.9 \pm 2\%$ for chemotherapy ($p = 0.09$). This increase in TRM was associated with a larger fraction of GO patients with prolonged time to neutrophil recovery in intensification II. Furthermore, this increase in TRM offset gains from the improved relapse rate, and thus no difference in OS was observed between the treatment arms in analyses of all patients. On subgroup analysis, high-risk

patients had improved OS and decreased relapse risk (26). Children with CD33-high AML treated with GO and chemotherapy on AAML0531 had disease-free survival rates similar to children with CD33-low AML, suggesting that GO treatment ameliorated the negative outcomes generally associated with high CD33 expression (27). These data highlight the potential for improved treatment efficacy with CD33-targeted agents for certain subgroups of patients, particularly in the setting of high CD33 antigen expression.

Gemtuzumab ozogamicin was withdrawn voluntarily from the U.S. market in 2010 due to concerns for increased induction mortality and lack of efficacy based upon preliminary data from the SWOG-106 trial. Later analyses noted unusually low control arm induction mortality and discordant anthracycline doses between the treatment arms, however, prompting questions regarding the validity of the decision for GO withdrawal (28, 29). More recent mature clinical trial data demonstrate improved outcomes with GO treatment in children and adults with *de novo* AML, particularly in those with favorable cytogenetic characteristics (26, 30–32). As above, GO-treated children treated on AAML0531 did not experience higher induction or overall toxic mortality in comparison to non-GO-treated children (26). A compassionate-use trial for adults and children (≥ 3 months of age) with relapsed/refractory AML or APL is currently open in the U.S. (NCT01869803) (33). While GO may return to the therapeutic armamentarium in the U.S. for pediatric and adult AML, additional evaluation will likely be required to determine its most appropriate implementation (29, 34).

Alternative anti-CD33 humanized antibody-drug conjugates, such as SGN-CD33A, are under current Phase 1 evaluation in adults with AML given very promising data from initial preclinical testing (NCT01902329) (33). SGN-CD33A is conjugated to a pyrrolobenzodiazepine dimer via a protease-cleavable linker, which has been reported to provide greater drug delivery and stability. Preclinical testing of SGN-CD33A *in vitro* in AML cell lines and *in vivo* in murine xenotransplantation models demonstrated superior leukemia cytotoxicity in comparison to GO. In addition, SGN-CD33A induced greater inhibition of leukemia proliferation and induction of apoptosis in xenograft models of drug-resistant AML (35).

Additional non-drug conjugate antibody approaches in preclinical and clinical testing for cancer include bispecific T cell engaging (BiTE) antibodies, which bind autologous T cells and redirect them specifically against tumor cell antigens. Such approaches have proven successful in early-phase testing for children and adults with leukemia, including the CD19/CD3 BiTE antibody blinatumomab (MT103) for B-precursor ALL (36, 37). Preclinical evaluation of the CD33/CD3 BiTE antibody AMG 330 demonstrated efficient *in vitro* lysis of CD33+ AML cell lines and primary blasts in the presence of human T cells, as well as *in vivo* efficacy in human AML xenograft models. Combination of AMG 330 with epigenetic-targeted therapies may have additional therapeutic efficacy. In preclinical studies, *in vitro* incubation of AML cells with panobinostat or azacitidine increased their CD33 expression, thereby increasing AMG 330-mediated cytotoxicity (38–40). BiTE antibodies for AML are not yet under clinical investigation.

Table 1 | Molecular therapeutic agents for pediatric acute myeloid leukemia (AML) in current clinical testing or late preclinical development and related trials for adult AML.

	Target(s)	Phase of testing	Clinical trial number (pediatric)	Clinical trial number (adult)
MONOCLONAL ANTIBODIES				
Brentuximab vedotin	CD30	1, 2	Not applicable	NCT01830777, NCT01461538
Gemtuzumab ozogamicin	CD33	1, 2, 3 (with chemotherapy)	NCT00476541, NCT00372593, NCT00070174 (completed)	NCT00091234, NCT00766116, NCT00893399
	CD33	Compassionate use	NCT01869803	NCT01869803
SGN-CD33A	CD33	1	Not applicable	NCT01902329
AMG 330	CD33	Not applicable	Not applicable	Not applicable
Lintuzumab-Ac225 (HuM195)	CD33	1, 2	Not applicable	NCT01756677, NCT00672165
90Y-BC8	CD45	1, 2 (with HSCT)	NCT00119366 (≥ 16 years)	NCT01300572, NCT00119366
IGN-523	CD98	1	Not applicable	NCT02040506
CSL362	CD123	1	Not applicable	NCT01632852
Ipilimumab	CTLA-4	1	Not applicable	NCT01757639
KB004	EphA3	1, 2	Not applicable	NCT01211691
TYROSINE KINASE INHIBITORS				
Lestaurtinib	JAK2, FLT3, TrkA	Not applicable	Not applicable	Not applicable
Midostaurin (PKC412)	CSFR1, FLT3, KIT, PDGFR	1, 2 (with chemotherapy)	NCT00866281	NCT01830361, NCT01883362, NCT01093573, NCT01846624, NCT01477606
Sorafenib	FLT3, Raf kinases, PDGFR, VEGFR	1, 2 (with chemotherapy or post-HSCT) 3	SJCRH RELHEM (NCT00908167) COG AAML1031 (NCT01371981)	NCT01861314, NCT01398501, NCT01534260
Sunitinib	CSF1R, FLT3, KIT, PDGFR, Raf kinases, RET, VEGFR	2	Not applicable	NCT01620216
KW-2449	FLT3	Not applicable	Not applicable	Not applicable
Quizartinib	CSFR1, FLT3, KIT, PDGFR	1, 2, 3	NCT01411267 (completed)	NCT02039726
PLX3397	CSFR1, FLT3, KIT	1, 2	Not applicable	NCT01349049
Linifanib (ABT-869)	FLT3, VEGFR	Not applicable	Not applicable	Not applicable
Crenolanib	FLT3, PDGFR	2	Not applicable	NCT01522469, NCT01657682
ASP2215	FLT3	1, 2	Not applicable	NCT02014558
Sirolimus	mTOR	1, 2 (with chemotherapy)	Not applicable	NCT01822015, NCT01869114, NCT00544999
Temsirolimus	mTOR	2 (with chemotherapy)	Not applicable	NCT01611116
Everolimus	mTOR	1 (with chemotherapy)	Not applicable	NCT01154439
Alisertib (MLN8237)	AURKA	1	Not applicable	NCT01779843
AMG 900	Pan-Aurora kinases	1	Not applicable	NCT01380756
AT9283	Pan-Aurora kinases	1	NCT01431664	Not applicable

(Continued)

Table 1 | Continued

	Target(s)	Phase of testing	Clinical trial number (pediatric)	Clinical trial number (adult)
PROTEASOME INHIBITORS				
Bortezomib	Proteasome	1, 2, 3 (with chemotherapy)	COG AAML1031 (NCT01371981)	NCT01127009, NCT01371981, NCT01861314, NCT01137747, NCT01204164, NCT01075425, NCT00410423
Carfilzomib	Proteasome	1	Not applicable	NCT01137747, NCT01204164
EPIGENETIC/DEMETHYLATING AGENTS				
Decitabine	Methyltransferases	1, 2 (with chemotherapy)	NCT01853228	Numerous
Azacitidine (5-azacytidine)	Methyltransferases	1 (with chemotherapy)	TACL T2011-002 (NCT01861002)	Numerous
Vorinostat	Histone deacetylases	1, 2	NCT01422499	Numerous
Panobinostat	Histone deacetylases	1	TACL T2009-012 (NCT01321346)	Numerous
EPZ-5676	DOT1L methyltransferase	1	Not applicable	NCT01684150
JQ1	Brd4	Not applicable	Not applicable	Not applicable
OXT015	Brd2/3/4	1	Not applicable	NCT01713582
SELECTIVE INHIBITORS OF NUCLEAR EXPORT				
KPT-330	CRM1	1	Not applicable	NCT01607892
CHIMERIC ANTIGEN RECEPTOR T CELL IMMUNOTHERAPY				
CART33	CD33	1, 2	NCT01864902	NCT01864902
CART123	CD123	Not applicable	Not applicable	Not applicable
Anti-Lewis-Y chimeric antigen receptor	Lewis-Y	1	Not applicable	NCT01716364

AURKA, aurora kinase A; COG, Children's Oncology Group; HSCT, hematopoietic stem cell transplantation; SJCRH, St. Jude Children's Research Hospital; TACL, Therapeutic Advances in Childhood Leukemia; NCT, ClinicalTrials.gov identifier.

Other antibody-based approaches for AML in early-phase clinical testing include targeting of surface proteins CD30, CD45, CD98, CD123, CTLA-4, or EphA3 (NCT01830777, NCT01756677, NCT02040506, NCT01632852, NCT01757639, NCT01211691) (33). Some of these approaches involve use of radiolabeled antibodies to increase leukemia cytotoxicity (NCT00672165, NCT01300572, NCT01756677) (33, 41). To our knowledge, such strategies have not been specifically evaluated in pediatric AML patients.

TYROSINE KINASE/FLT3 INHIBITORS

Somatic internal tandem duplication of the gene encoding the fms-like tyrosine kinase receptor-3 (*FLT3*-ITD) with a high mutant:wild-type allelic ratio (>0.4) or point mutations in the *FLT3* activation loop of the tyrosine kinase domain (*FLT3*-TKD) occur in 15–20% of pediatric AML patients (42). Similar to adults, children with *FLT3*-ITD AML respond poorly to conventional chemotherapy and have OS of 25–30% even with HSCT (42, 43). Salvage rates of patients with relapsed/refractory *FLT3*-ITD AML are particularly poor with 5-year OS <10% (44). Interestingly, *FLT3*-TKDs do not appear to confer worse outcomes in adults or children with AML (42, 45).

FLT3-ITD alterations disrupt the negative regulatory function of the *FLT3* protein and facilitate constitutive kinase activation, resulting in perturbed Ras/MAPK, PI3K/Akt/mTOR, and/or JAK/STAT signal transduction and arrest of apoptosis (46, 47). Preclinical studies demonstrate preferential sensitivity of *FLT3*-ITD AML to tyrosine kinase inhibitors (TKIs) that target the *FLT3* receptor. In the clinic, TKIs targeting mutant *FLT3* have been studied primarily in early-phase trials in adult patients. Modest efficacy of first-generation *FLT3* inhibitors (e.g., lestaurtinib, midostaurin, sorafenib, sunitinib) in adults with relapsed AML has been reported, both as monotherapy and in conjunction with cytotoxic chemotherapy (48–52). Many of these first-generation inhibitors are known to target other kinases and wild-type *FLT3* protein to some degree and were not designed to inhibit *FLT3* preferentially (50, 53–55). Subsequently, TKIs targeting *FLT3* more specifically (e.g., KW-2449, PLX3397, quizartinib, linifanib) were developed and are in various stages of preclinical and clinical evaluation as described below (48, 56–59). However, resistance to TKI therapy is well-described and has been attributed to a variety of mechanisms, including development of mutations that interfere structurally with drug binding, alterations in cell survival and signaling pathways, and bone marrow microenvironment factors promoting

leukemic survival (60–62). Consequently, third-generation FLT3 TKIs (e.g., crenolanib, ASP2215) have been designed to overcome resistance and have demonstrated preclinical efficacy in drug-resistant *FLT3*-ITD AML with acquired mutations (63–65). These selective FLT3 TKIs are also in early-phase clinical testing for adults with AML (NCT01522469, NCT01657682, NCT02014558) (33). Despite their favorable pharmacokinetic and pharmacodynamic (PD) properties, the majority of next-generation FLT3 TKIs have thus far demonstrated relatively limited anti-AML clinical efficacy as monotherapy and will likely require combination with cytotoxic chemotherapy to achieve maximal therapeutic benefit (55, 66–69).

FLT3 inhibition strategies remain incompletely elucidated and, to date, have undergone relatively limited evaluation in children. Initial preclinical studies demonstrated preferential cytotoxicity of pediatric *FLT3*-ITD AML specimens incubated *in vitro* with lestaurtinib (formerly CEP-701) (70). Lestaurtinib has been better studied clinically in infants with *de novo* *MLL*-rearranged B-precursor acute lymphoblastic leukemias (ALLs), which over-express the wild-type FLT3 receptor (71, 72). Lestaurtinib remains under investigation for infant ALL in the current COG Phase 3 trial AALL0631 (NCT00557193) (33, 73). The multi-kinase inhibitor sorafenib was initially evaluated in the COG Phase 1 trial ADVL0413 in children with advanced solid tumors and leukemias; two children with relapsed/refractory *FLT3*-ITD AML achieved \geq CR2 with sorafenib monotherapy on this trial and subsequently underwent HSCT (74). In an institutional case series, three children with relapsed/refractory AML treated with sorafenib and cytotoxic chemotherapy also achieved remission (75). Similarly, 8 of 11 children with relapsed/refractory AML (5 *FLT3*-ITD and 3 *FLT3*-wild-type) treated on a Phase 1 trial at SJCRH with sorafenib, clofarabine, and cytarabine achieved CR or CR with incomplete blood count recovery (CRi) (76).

Recent studies have demonstrated tolerability and improved induction remission in younger adults with *de novo* *FLT3*-ITD AML treated with sorafenib and chemotherapy, although OS did not differ between the chemotherapy and chemotherapy/sorafenib arms (77). Similarly, a Study Alliance Leukemia trial recently demonstrated no improvement in EFS or OS with sorafenib addition to induction and consolidation chemotherapy for elderly patients with AML (78). However, given the earlier promising clinical data specifically in pediatric AML, the combination of sorafenib and cytotoxic chemotherapy for children with *FLT3*-ITD AML is currently under prospective investigation in the COG Phase 3 trial AAML1031 (NCT01371981) (33). In this trial, children with *FLT3*-ITD AML are non-randomly assigned to treatment with sorafenib in combination with standard chemotherapy for assessment of feasibility (stage 1) and efficacy (stage 2), then taken to HSCT with the best available donor. Interim analyses of AAML1031 data have demonstrated the feasibility and safety of sorafenib with chemotherapy in stage 1, and stage 2 efficacy studies are now underway. A 1-year sorafenib maintenance phase for patients post-HSCT (or post-chemotherapy completion for non-transplanted patients) is underway in AAML1031 based in part upon safety and outcome data from a multi-institutional pilot study (33, 79). Similar sorafenib maintenance studies for adult AML patients are also in progress in the U.S. and in Europe (NCT01398501, NCT01578109, and JA Pollard, personal

communication) (33). Although not under current clinical investigation, sorafenib's c-kit inhibition properties may also have utility for children with *KIT*-mutant AML, which comprises 30% of pediatric core binding factor AML (80).

Studies of the more FLT3-selective second-generation FLT3 TKIs, particularly quizartinib (formerly AC220), have garnered considerable attention for treatment of adults with *FLT3*-ITD AML. Recent Phase 1 and 2 studies of quizartinib in adults with relapsed/refractory AML demonstrated acceptable toxicity profiles and preferential responses of *FLT3*-ITD patients (44, 81). A first-in-children Phase 1 study of quizartinib with chemotherapy for children with relapsed/refractory leukemias was recently conducted via the Therapeutic Advances in Childhood Leukemia (TACL) consortium Phase 1 trial TACL 2009-004 (NCT01411267) (33, 82). The combination regimen was well-tolerated at all doses of quizartinib studied, and near-complete inhibition of phosphorylated FLT3 was observed via PD assays. Three of the six children with *FLT3*-ITD AML achieved CR or CRi, and one of the eight children with *FLT3*-wild-type AML achieved CR (82).

Based upon promising preclinical data in leukemia and clinical testing in solid tumor patients with platelet-derived growth factor receptor (PDGFR) mutations (65, 83), a Phase 2 study of the third-generation FLT3/PDGFR inhibitor crenolanib in adults with AML is also in progress (NCT01522469) (33). Early PD analyses of blood samples from patients enrolled on this trial demonstrate potent FLT3 inhibition with crenolanib treatment (64). Preclinical data also suggest that crenolanib may have efficacy against resistance-conferring *FLT3* point mutations that emerge during TKI therapy, including *FLT3* D835 point mutations. Limited preclinical evaluation of crenolanib incubated with primary leukemia specimens from children with TKI-resistant *FLT3*-ITD/*FLT3* D835 AML demonstrated moderate *in vitro* anti-leukemic activity (63). Most recently, the third-generation FLT3 inhibitor ASP2215 is under Phase 1/2 evaluation in adults with relapsed/refractory AML (NCT02014558) (33). Crenolanib and ASP2215 have not been evaluated clinically in children at this time.

Other TKI approaches for poor-risk adult AML are also under clinical investigation (84). Constitutive activation of the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signal transduction cascade has been well-documented in AML, and PI3K/Akt/mTOR signaling activation often occurs as a downstream sequela of *FLT3*-ITD alterations (85–87). Development of new approaches to inhibit aberrant PI3K pathway signaling is of intense preclinical and clinical interest for AML biologists and clinicians. The mTOR inhibitor rapamycin (also known as sirolimus) and its derivatives are directly cytotoxic to primary AML samples *in vitro* and have proven synergistic with AML-directed cytotoxic chemotherapy *in vivo* in mouse models (88–90). Several early-phase clinical trials in the U.S. and Europe are currently evaluating mTOR inhibition (e.g., sirolimus, temsirolimus, everolimus) in combination with cytotoxic chemotherapy in adults with *de novo* AML (NCT01611116, NCT01154439, NCT01822015, NCT01869114) (33). Although mTOR inhibition in combination with cytotoxic chemotherapy for children with relapsed ALL is under investigation via various consortia (NCT01403415, NCT01523977, NCT01614197) (33), such regimens have not been evaluated in pediatric AML.

Inhibition of the Aurora family of kinases is also under study in patients with relapsed/refractory leukemias. Aurora kinases are critical proteins involved in normal cellular mitosis. Aurora kinase overexpression has been documented in AML and hypothesized to contribute to leukemogenesis (91, 92). Preclinical testing has demonstrated preferential sensitivity of AML cell lines and primary AML specimens to Aurora A and Aurora B kinase inhibitors (91, 92). These agents may have particular therapeutic relevance for patients with acute megakaryoblastic leukemia (AMKL) based upon preclinical data demonstrating increased megakaryocyte polyploidization and AMKL cytotoxicity *in vitro* and *in vivo* with the Aurora kinase A inhibitor alisertib (MLN8237) (93). Clinical trials are in progress to evaluate the safety and/or efficacy of Aurora kinase inhibition as monotherapy or in conjunction with chemotherapy in adults with AML (NCT01779843) (33). Early-phase trial investigation of Aurora kinase inhibitors in children with relapsed/refractory leukemias is also underway in the United Kingdom and the U.S. (NCT01431664, NCT01154816) (33).

PROTEASOME INHIBITORS

The proteasome inhibitor bortezomib has been investigated in the treatment of various malignancies and has demonstrated single-agent efficacy in multiple myeloma. Bortezomib is hypothesized to deplete selectively leukemia-initiating cells and may also augment effects of cytotoxic chemotherapeutic agents (94, 95). Bortezomib has been evaluated specifically in adults with AML in the relapse and *de novo* settings, where it has been reasonably well-tolerated and has demonstrated efficacy in combination with other agents (96–98). Bortezomib and other proteasome inhibitors (e.g., carfilzomib) are under active investigation for adult AML in combination with cytotoxic or demethylating agents in several current clinical trials (NCT01127009, NCT01371981, NCT01861314, NCT01137747, NCT01204164) (33).

Bortezomib was first studied in children with relapsed/refractory solid tumors and leukemia in the COG and TAEL consortia (99–102). The subsequent COG Phase 2 trials AALL07P1 and AAML07P1 evaluated bortezomib with intensive re-induction chemotherapy specifically in children with relapsed/refractory ALL and AML, respectively (103, 104). These studies demonstrated safety and tolerability of combination regimens in children with relapsed/refractory leukemias. Among the 38 children with AML treated on AAML07P1 and evaluable for efficacy, 11 CR, 3 CR with incomplete platelet recovery (CRp), and 5 CR with incomplete neutrophil recovery (CRi) were achieved, although OS was not improved with bortezomib addition in comparison to survival data from historical controls (104). Bortezomib in combination with standard AML chemotherapy is currently under investigation for children with *de novo* AML vs. standard AML chemotherapy in the randomized COG Phase 3 trial AAML1031 (NCT01371981) (33).

EPIGENETIC/DEMETHYLATING AGENTS

Genes that regulate DNA methylation and demethylation (e.g., *DNMT3A*, *IDH1* and *IDH2*, *TET2*) are commonly mutated in adult AML and myelodysplastic syndromes (MDS), although the frequency of these mutations appears much lower in pediatric AML (105–109). DNA methyltransferase inhibitors

(e.g., decitabine, azacitidine/5-azacytidine) are in clinical testing for adults with AML and MDS, and a recent trial demonstrated improved response rates in adults with *DNMT3A*-mutant AML who were treated with decitabine (110). Initial testing of demethylating agents in children with relapsed/refractory AML was performed through the COG, and a recent single-institution study also reported CRs in three of eight children with relapsed/refractory AML treated with decitabine monotherapy (111, 112). Phase 1 studies of azacitidine or decitabine with chemotherapy for children with relapsed/refractory acute leukemias are planned (NCT01861002, NCT01853228) (33).

An additional promising epigenetic strategy for pediatric AML involves inhibition of the DOT1L histone methyltransferase. Preclinical studies of *MLL*-rearranged leukemias have demonstrated a critical role of this enzyme for leukemogenesis, as well as preferential leukemia cytotoxicity with DOT1L inhibition (e.g., EPZ004777) (113–115). A first-in-human Phase 1 study of the DOT1L inhibitor EPZ-5676 for adults with relapsed/refractory *MLL*-rearranged leukemias recently opened for accrual (NCT01684150) (33).

Another epigenetic strategy involves inhibition of histone deacetylases with agents such as valproic acid, vorinostat, or panobinostat. Vorinostat has proven potentially promising in combination with cytotoxic chemotherapy via early-phase clinical trials for adults with *de novo* AML or MDS (116, 117). A Phase 1/2 study of vorinostat in children with relapsed/refractory cancer, including leukemia, is in progress in Europe (NCT01422499) (33). A Phase 1 study of vorinostat with azacitidine and chemotherapy for children with relapsed ALL is underway via the TAEL consortium (NCT01483690) (33), but this combination is not under current investigation in pediatric AML. Early clinical data from adults with AML or MDS treated with vorinostat and demethylating agents demonstrate excellent hematologic responses in many patients (118–121). In one study, clinical responses of patients treated with vorinostat and azacitidine correlated with high pre-treatment methylation levels and demethylation and acetylation during therapy, while patients without evidence of basal hypermethylation or post-treatment demethylation did not respond (119). A Phase 1 trial of panobinostat for children with relapsed/refractory hematologic malignancies is also underway via TAEL (NCT01321346) (33). The role of such epigenetic therapies for children with AML remains to be elucidated fully, but early clinical data in adults and children with AML appear encouraging.

The bromodomain and extra-terminal (BET) family of proteins recognize acetylated lysine residues of histone proteins and modulate gene expression via recruitment of transcriptional regulators (122). Inhibition of BET proteins, particularly of Brd4, has demonstrated remarkable anti-tumor activity in a variety of human cancers. Preclinical evaluation of the Brd4 inhibitor JQ1 in AML demonstrated potent *in vitro* and *in vivo* inhibition of leukemia proliferation and elimination of leukemia-initiating cells, likely via suppression of *MYC* (123, 124). Combination approaches of Brd4 inhibition with TKIs, HDAC inhibitors, or cytotoxic chemotherapy have demonstrated preclinical efficacy in subtypes of AML and remain under active study (125, 126). The preclinical efficacy of the BRD2/3/4 inhibitor OXT015 in AML was also recently reported. *In vitro* incubation of primary AML cells with OXT015 resulted in

cell cycle arrest and induction of apoptosis (127). A Phase 1 trial of OTX015 for adults with hematologic malignancies is ongoing in Europe (NCT01713582) (33).

SELECTIVE INHIBITORS OF NUCLEAR EXPORT

Localization of cytosolic proteins to the nucleus is critical for normal cellular function. However, the acquired ability of malignant cells to export key nuclear proteins to the cytoplasm is hypothesized to be a major mechanism of treatment resistance. Selective inhibitors of nuclear export, particularly agents targeting the chromosome region maintenance 1 export protein (CRM1, also known as XPO1), have been evaluated in the preclinical setting for various hematologic malignancies, including AML (128, 129). Testing of the CRM1 inhibitors KPT-185 and KPT-251 demonstrated *in vitro* activity against human AML cell lines harboring various genetic alterations and *in vivo* efficacy in AML cell line xenotransplantation models, likely via G1 cell cycle arrest and induction of apoptosis (128, 130). A Phase 1 study of the CRM1 inhibitor KPT-330 is in progress for adults with advanced hematologic malignancies (NCT01607892) (33).

CHIMERIC ANTIGEN RECEPTOR T CELL IMMUNOTHERAPY

Rapid progress has been made recently in the development of cancer immunotherapy using human T cells genetically engineered with synthetic chimeric antigen receptors (CARs) to target tumor antigens (131). Current immunotherapeutic approaches for childhood leukemias, including CAR T cell therapy, have been specifically delineated by our colleagues elsewhere in this review series (132). While particular preclinical and early clinical progress has been made with anti-CD19 CAR T cell strategies for B-cell malignancies (133–135), development of anti-AML CAR T cell immunotherapies for the clinic has proven more difficult. Similar to monoclonal antibody therapies for AML (e.g., GO), the paucity of well-characterized, truly leukemia-specific surface antigens in AML has necessitated consideration of CAR T cell AML-targeting strategies that will likely deplete normal hematopoietic cells via “on target/off tumor” effects. Careful consideration of both the leukemia cytotoxicity and the potential for CAR T cell-mediated myeloablation is essential prior to translation of these approaches to the clinic for adults and children with AML.

To date, several candidate AML-associated antigens for CAR T cell approaches have been selected for investigation, including CD33, CD44, CD123, and Lewis-Y (136–141). Preclinical studies of the various anti-AML CAR T cells have generally demonstrated potent *in vitro* cytotoxicity against human AML cell lines and primary specimens (138, 141). Some groups have further observed *in vivo* efficacy of anti-AML CARs using human AML xenograft models (138, 140, 141). However, some reports have noted depletion of normal CD34+ hematopoietic cells by the anti-AML CAR T cells, highlighting the myeloablative potential of such immunotherapeutic approaches for AML (139–141). CAR T cell therapy approaches for AML remain primarily in the preclinical testing phase in the U.S. and Europe, although Phase 1 trials of anti-CD33 CAR T cell therapy (NCT01864902; children ≥ 5 years of age eligible) and anti-Lewis-Y CAR T cell therapy (NCT01716364; patients ≥ 18 years of age) for patients

with relapsed/refractory AML were recently opened in China and Australia, respectively (33).

TOWARD PRECISION MEDICINE FOR PEDIATRIC AML

Despite intensive multi-agent chemotherapy, more than one-third of children with AML will die of chemorefractory or relapsed disease. Further therapy intensification with traditional cytotoxic drugs is unlikely to be tolerated given the current dose intensity of AML chemotherapy. Therefore, new molecular therapeutic approaches that target the AML stem cell responsible for leukemia initiation and progression and that have minimal impact upon normal tissues are needed. Implementation of such targeted strategies will be more likely successful when used in rationally selected combination regimens, which may allow for lower drug dosing (thereby minimizing overlapping toxicities), decrease acquired drug resistance, and increase potential for additive or synergistic cytotoxicity. At a genomics level, significant collaborative efforts are ongoing via the SJCRH–Washington University Pediatric Cancer Genome Project, the National Cancer Institute’s therapeutically applicable research to generate effective treatments (TARGET) AML Project, and other consortia to delineate more precisely various genetic subgroups of pediatric AML and to identify “actionable” lesions for molecularly targeted therapies (8, 142, 143).

One important consideration will be the degree to which results should be extrapolated from adult clinical trial data for children with AML given the inherent, but incompletely understood, differences in biology and in therapeutic responses. Results from early-phase clinical trials of new agents and the number of novel drugs in preclinical development are encouraging, but significant challenges persist in drug development for pediatric oncology. Evaluation of new agents in children with relapsed leukemia in early-phase clinical trials remains particularly challenging given the limited availability of novel drugs for pediatric studies. Moreover, study accrual can be challenging due to rapid disease progression in this population that hinders enrollment. Evaluation of meaningful responses in this setting is also difficult given the use of stringent disease response definitions that limit the number of cycles of therapy administered despite drug mechanisms that may require multiple courses of treatment to achieve optimal response. Given the relative rarity of childhood AML, its biologic heterogeneity, and the prospect of tailored therapeutics for ever-smaller genetic subgroups, collaborative trials across pediatric oncology trial consortia may be a more efficient means by which to identify new molecularly targeted agents for children with specific biologic subtypes of AML. Nonetheless, investigation of promising new therapies is essential to decrease relapse and improve cure for children with AML, and enthusiasm remains high for the development of molecularly targeted approaches in this emerging era of precision medicine.

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