Novel agents for the treatment of childhood acute leukemia

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***Abstract*:** Together, acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) make up approximately one-third of all pediatric cancer diagnoses. Despite remarkable improvement in the treatment outcomes of these diseases over the past several decades, the prognosis for certain high-risk groups of leukemia and for relapsed disease remains poor. However, recent insights into different types of 'driver' lesions of leukemogenesis, such as the aberrant activation of signaling pathways and various epigenetic modifications, have led to the discovery of novel agents that specifically target the mechanism of transformation. In parallel, emerging approaches in cancer immunotherapy have led to newer therapies that can exploit and harness cytotoxic immunity directed against malignant cells. This review details the rationale and implementation of recent and specifically targeted therapies in acute pediatric leukemia. Topics covered include the inhibition of critical cell signaling pathways [BCR-ABL, FMS-like tyrosine kinase 3 (FLT3), mammalian target of rapamycin (mTOR), and Janus-associated kinase (JAK)], proteasome inhibition, inhibition of epigenetic regulators of gene expression [DNA methyltransferase (DNMT) inhibitors, histone deacetylase (HDAC) inhibitors, and disruptor of telomeric signaling-1 (DOT1L) inhibitors], monoclonal antibodies and immunoconjugated toxins, bispecific T-cell engaging (BiTE) antibodies, and chimeric antigen receptor-modified (CAR) T cells.

Keywords: ALL, AML, blinatumomab, CAR T-cells, carfilzomib, EPZ-5676, FLT3, moxetumomab

Introduction

Remarkable progress has been made in the past several decades in the treatment of childhood acute lymphoblastic leukemia (ALL), with 5-year survival rates now approaching 90% [Hunger *et al.* 2012]. This success has been achieved with a risk stratification design incorporating minimal residual disease (MRD) status, and the intensification of cytotoxic chemotherapy for those at highest risk [Pui *et al.* 2011a]. However, up to 20% of children with ALL will be refractory to treatment or relapse following treatment, and the survival rate for relapsed ALL remains poor. In addition, although the majority of children with acute myeloid leukemia (AML) will achieve remission with conventional chemotherapy, less than 60% will be long-term survivors. Cytotoxic therapy intensification has been maximized in the treatment of AML, highlighting the need for additional, targeted novel therapies in this disease [Pui *et al.* 2011b]. Targeted therapies in pediatric leukemia are largely unproven to date, with the clear exceptions of tyrosine kinase inhibitors (TKIs) in BCR-ABL (Philadelphia chromosome) positive leukemia [Schultz *et al.* 2009], and all-trans retinoic acid (ATRA) in acute promyelocytic leukemia (APML) with PML-RARa fusions [Tallman, 2004]. Challenges have included the effective incorporation of novel agents into current chemotherapy regimens, the development of resistance to targeted therapies, and failure to eradicate the leukemia stem cell (LSC) population in an individual disease. In this article, we summarize recent and developing novel drugs for pediatric acute leukemia, review open and recently completed clinical trials using these agents (listed in Table 1), and discuss plans to implement these therapies into pediatric ALL or AML treatment regimens.

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Tyrosine kinases (TKIs)

BCR-ABL

Among the successful stories of targeted therapies in pediatric acute leukemia is the introduction of the TKI imatinib into upfront therapy for Philadelphia chromosome positive (Ph+) ALL patients. The Children's Oncology Group (COG) trial AALL0031 (2002–2006) incorporated imatinib into an upfront, intensive chemotherapy backbone for Ph+ ALL pediatric patients. Initial results from this trial demonstrated a 3-year event-free survival (EFS) of 88%, doubling that of historical controls [Schultz *et al.* 2009]. In addition, longer follow-up revealed that Ph+ ALL patients receiving chemotherapy with continuous imatinib had similar 5-year EFS rates to patients undergoing either related or unrelated donor hematopoietic stem-cell transplant (HSCT; 71%, 64% and 63%, respectively; $p=0.77$) [Schultz *et al.* 2014]. Retrospective analysis of patients who relapsed after treatment demonstrated remission reinduction rates similar to other high-risk non-Ph+ ALL patients treated on contemporaneous trials, allowing these patients to proceed to HSCT as salvage therapy [Schultz *et al.* 2014]. Imatinib was FDA-approved for the treatment of Ph+ ALL in children in 2013. However, a well-known mechanism of resistance to TKI therapy is the outgrowth of resistant clones, often mediated through the development of point mutations in the kinase domain of ABL. In a recent review of 272 adult patients with relapsed Ph+ ALL, 70% harbored a kinase domain point mutation, including T315I, E255K and Y253H [Soverini *et al.* 2014]. Interestingly, kinase point mutations seem to be less common in pediatric Ph+ ALL at relapse, although they have been demonstrated to occur [Chang *et al.* 2012].

Dasatinib, a second-generation TKI, replaced imatinib in the most recent COG trial AALL1122 for Ph+ ALL patients, after COG phase I/II trial AALL0622 demonstrated good tolerability and rapid efficacy of dasatinib in combination with chemotherapy. There is *in vitro* evidence that dasatinib has superior central nervous system (CNS) penetration compared with imatinib [Porkka *et al.* 2008]. Dasatinib is effective against many resistant mutations, with the exception of point mutation T315I [Talpaz *et al.* 2006]. Nilotinib is another second-generation TKI that has been less studied in both adults and pediatrics, but some reports show efficacy against certain dasatinib-resistant mutations, although not T315I [Jabbour *et al.* 2008; Sekimizu *et al.* 2013]. A phase II COG study is investigating the efficacy of nilotinib in pediatric chronic myeloid leukemia (CML), and a multi-institutional phase I study of nilotinib is open for pediatric patients with CML or relapsed/refractory Ph+ ALL (Table 1). The third-generation TKI, ponatinib, is active against the increasingly clinically significant mutation T315I, but toxicities of arterial thrombosis risk were documented [Cortes *et al.* 2013], temporarily halting its development in clinical trials. As of January 2014, ponatinib is FDA-approved for adults with Ph+ leukemia resistant to other TKIs, now carrying the additional warning of thrombosis. Other classes of kinase inhibitors are being explored in adult Ph+ leukemia in an attempt to prevent the development of resistance, such as the Janus-associated kinase (JAK) inhibitor, ruxolitinib, in combination with nilotinib [ClinicalTrials.gov identifiers: NCT01702064 and NCT01914484].

FMS-like tyrosine kinase 3 (FLT3)

FMS-like tyrosine kinase 3 (FLT3) is a receptor tyrosine kinase expressed on human $CD34^+$ hematopoietic stem and early progenitor cells, and FLT3 signaling is central to cell proliferation and differentiation [Small *et al.* 1994]. FLT3 is aberrantly expressed on the majority of leukemic blasts regardless of CD34 expression [Carow *et al.* 1996]. Of note, the most consistently overexpressed gene in mixed lineage leukemia-rearranged (MLL-r) infant ALL is wild-type FLT3 [Armstrong *et al.* 2002]. Also, mutations of FLT3 occur in 20–25% of pediatric AML patients, and result in ligand-independent constitutive activation of the receptor [Kondo *et al.* 1999; Meshinchi *et al.* 2001]. Roughly two-thirds of these mutations are internal tandem duplications (ITD) of the juxtamembrane domain of the gene, and the remaining one-third are point mutations of the tyrosine kinase domain (TKD) [Meshinchi *et al.* 2001; Yamamoto *et al.* 2001]. Multiple studies have documented decreased overall survival and increased rate of relapse in FLT3-ITD mutant AML [Iwai *et al.* 1999; Kondo *et al.* 1999; Meshinchi *et al.* 2001]. In one study, children with ITD mutations had 8-year overall survival (OS) and EFS rates of 13% and 7%, respectively, compared with an OS of 50% and EFS of 44% for children without ITD mutations [Meshinchi *et al.* 2001]. A large retrospective

Table 1. Active and recently completed clinical trials with novel agents in pediatric acute leukemia.

Drug	Target	Disease	Phase	Clinical trial identifier(s)	
Dasatinib	BCR-ABL	ALL (Ph+)	II	NCT01460160	*completed
				COG AALL1122	
Nilotonib	BCR-ABL	ALL, CML (Ph+)	I	NCT01077544	
				CAMN107A2120	
Lestaurtinib	FLT3	Infant ALL	III	NCT00557193	*completed
		AML	I	COG AALL0631	
				NCT00469859	*completed
				COG AAML06P1	
Midostaurin	FLT3	Infant ALL, AML	I/II	NCT00866281	
Quizartinib	FLT3	ALL, AML	I	NCT01411267	*completed
				TACL 2009-004	
Sorafenib	FLT3, other RTKs	AML	III	NCT01371981	
		Infant ALL, AML, MDS	I	COG AAML1031	
		ALL, AML	I	NCT00908167	
				RELHEM	
				NCT00665990	
				ANGIO1	
Rapamycin	mTOR	ALL, NHL	I	NCT01658007	
		ALL	III	2012-0361	
				NCT00382109	*completed
				COG ASCT0431	
Temsirolimus	mTOR	ALL, NHL	I	NCT01403415	
				COG ADVL1114	
Everolimus	mTOR	ALL	I	NCT01523977	
		ALL	I/II	11-237	
				NCT00968253	
				2009-0100	
Ruxolitinib	JAK 1/2	ALL, AML	I	NCT01164163	*completed
				COG ADVL1011	
Bortezomib	Proteasome	ALL, AML	I	NCT00077467	*completed
		AML	II	COG ADVL0317	
		ALL	II	NCT00666588	*completed
		AML	III	COG AAML07P1	
				NCT00873093	*completed
				COG AALL07P1	
				NCT01371981	
				COG AAML1031	
Azacitidine	DNMT	ALL, AML	I	NCT01861002	*completed
				TACL 2011-002	
Vorinostat	HDAC	ALL	I/II	NCT01483690	
				TACL 2009-003	
Panobinostat	HDAC	ALL, AML, HL, NHL	I	NCT01321346	
				TACL 2009-012	
EPZ-5676	DOT1L	ALL, AML (MLL-r)	IIb	NCT02141828	
Gemtuzomab	CD33	AML	III	NCT00372593	*completed
				COG AAML0531	
Epratuzumab	CD22	CD22+ ALL	I/II	NCT00098839	*completed
		ALL	II	ADVL04P2	
				NCT01802814	

(Continued)

Table 1. (Continued)

Drug	Target	Disease	Phase	Clinical trial identifier(s)	
				IntReALL SR 2010	
Inotuzumab	CD22	ALL	I/II	NCT01134575	*completed
				2009-0872	
Moxetumomab	CD22	ALL or NHL	I	NCT00659425	
			II	CAT-8015-1004	
		ALL		NCT02227108	
				CAT-8015-1036	
Blinatumomab	CD3, CD19	ALL	I/II	NCT01471782	
		ALL	III	MT103-205	
				NCT02101853	
				COG AALL1331	
CAR T-cells	CD19	CD19+ ALL / lymphoma	I/IIa	NCT01626495	
		CD19+ ALL / lymphoma	II	10-007706	
		CD19+ ALL	I	NCT01593696	
		CD19+ ALL	I	120112, 12-C-0112	
		CD19+ ALL	I/II	NCT01860937	
				13-052	
				NCT01683279	
				PLAT-01	
				NCT02028455	
				PLAT-02	

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Ph+, Philadelphia chromosome positive; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; MLL-r, mixed lineage leukemia gene rearranged; DNMT, DNA methyltransferase; HDAC, histone deacetylase; DOT1L, Disruptor of telomerase-1; CAR T-cells, chimeric antigen receptor.

review determined that an ITD allelic ratio of 0.4 or higher identified the highest risk group with the worst prognosis, whereas children with allelic ratios <0.4 had similar outcomes to those with wild type FLT3 [Meshinchi *et al.* 2006]. These data provide strong rationale for the use of FLT3 inhibitors in pediatric acute leukemia.

The FLT3 inhibitor lestaurtinib (CEP-701) has shown modest efficacy in adult trials as monotherapy [Smith *et al.* 2004; Knapper *et al.* 2006] or in combination with chemotherapy [Levis *et al.* 2011]. In pediatrics, a phase I trial of lestaurtinib with chemotherapy for relapsed/refractory AML has been completed, though clinical data are not yet published. However, at each dose level, five of six patients tested had >80% inhibition of FLT3 phosphorylation at the majority of lestaurtinib trough time points. Recently completed COG phase III trial AALL0631 investigated lestaurtinib in combination with chemotherapy for newly diagnosed infant ALL. Intermediate-risk (MLL-r and >90 days old) and high-risk (MLL-r and <90 days old) infants were randomized to receive lestaurtinib after induction chemotherapy. Trial efficacy results are pending.

Midostaurin (PKC412) is a multi-TKI that has activity against FLT3, and early phase adult clinical trials showed hematological responses in patients with mutant FLT3 [Stone *et al.* 2005; Fischer *et al.* 2010]. In pediatrics, a phase I/II clinical trial open in Europe and some US centers is currently recruiting MLL-r infant ALL and FLT3-mutant AML patients to receive midostaurin as a single agent. Laboratory correlatives include evaluation of FLT3 phosphorylation before and after receiving drug.

Quizartinib, or AC220, is a far more potent and selective FLT3 inhibitor than lestaurtinib and midostaurin. In a phase II study of quizartinib as monotherapy in relapsed/refractory adults with FLT3-ITD mutant AML, a complete response (CR) or a complete response with incomplete blood count recovery (CRi) was induced in 9 of 17 patients (53%) [Levis *et al*. 2012]. Current adult studies are investigating quizartinib in combination with chemotherapy. In pediatrics, a recently completed Therapeutic Advances in Childhood Leukemia and Lymphoma (TACL) pilot study investigated quizartinib in combination with cytarabine and etoposide in patients with MLL-r ALL or relapsed/refractory AML. Laboratory correlatives showed near-complete inhibition of FLT3 phosphorylation; importantly, four of six patients with FLT3-ITD mutant AML achieved a CR or CRi, and the other two ITD patients had stable disease [Cooper *et al*. 2013].

Sorafenib, a multi-TKI with activity against FLT3, induced clinical responses specifically in ITD-mutant cases of relapsed/refractory AML in three independent phase I adult trials [Zhang *et al*. 2008; Crump *et al*. 2010; Pratz *et al*. 2010]. A phase I/II trial in newly diagnosed adult AML combined sorafenib with cytarabine and idarubicin and impressively, 38 of 51 (75%) patients achieved a CR, including 14 of 15 FLT3-ITD patients [Ravandi *et al*. 2010]. In 12 pediatric patients with relapsed/refractory AML, 5 of 5 FLT3-ITD and 3 of 7 wild type FLT3 patients achieved a CR or CRi with sorafenib in combination with clofarabine and cytarabine [Inaba *et al*. 2011]. Given these encouraging reports, the current COG phase III trial for newly diagnosed pediatric AML nonrandomly assigns ITD-mutant patients with high allelic ratios (>0.4) to receive sorafenib in combination with chemotherapy, and includes a maintenance phase with single agent sorafenib for 1 year. There are also two phase I studies open at St. Jude Children's Research Hospital, combining sorafenib with chemotherapy in patients with refractory hematological malignancies.

Serine/threonine kinases

mTOR

Mammalian target of rapamycin (mTOR) is a serine/threonine kinase, and is centrally integrated in several key signal transduction pathways critical to cell growth and proliferation. Aberrant activation of the mTOR pathway has been demonstrated in multiple tumor types, and inhibition of mTOR by the macrolide rapamycin (sirolimus) or one of its analogs (temsirolimus, everolimus) has shown antitumor activity in preclinical models and in early phase clinical trials.

Constitutive activation of the mTOR pathway has been demonstrated in the majority of cases of AML [Min *et al*. 2003; Xu *et al*. 2003; Recher *et al*. 2005b]. Rapamycin treatment causes G0/G1 cell cycle arrest in AML cell lines, and inhibits the clonogenic properties of AML patient samples without significantly affecting healthy donor $CD34^+$ bone marrow (BM) cells [Recher *et al*. 2005a]. Rapamycin induced clinical responses in 4 of 9 relapsed/refractory adult AML patients when given as a single agent for a 28-day course [Recher *et al*. 2005b]. A phase I/II study of single-agent everolimus in adults with relapsed/refractory leukemia demonstrated good tolerability and a modest clinical response [Yee *et al*. 2006]. Currently, there are no ongoing trials investigating mTOR inhibitors in childhood AML.

Rapamycin has also been shown to have activity in pre-B cell ALL cell lines, primary patient samples, and a xenograft model [Brown *et al*. 2003; Maude *et al*. 2012]. mTOR inhibitors also synergize with methotrexate in pre-B ALL cells [Teachey *et al*. 2008]. In addition, rapamycin was shown to sensitize steroid-resistant malignant lymphoid cells to glucocorticoid-induced apoptosis via modulation of anti-apoptotic MCL1 [Wei *et al*. 2006], and could therefore have a role in overcoming glucocorticoid resistance, a known poor prognostic factor in ALL. These studies provide strong rationale to pursue mTOR inhibition in combination with chemotherapy in pediatric ALL, and there are multiple ongoing early phase clinical trials investigating these agents. A single-institution pilot trial at Cincinnati Children's hospital combines rapamycin with the mitoxantrone arm of UKALLR3 reinduction chemotherapy [Parker *et al*. 2010] in patients with relapsed ALL up to 30 years of age. The phase I COG study ADVL1114 is investigating temsirolimus in combination with a similar reinduction chemotherapy backbone in relapsed pediatric ALL patients. A multi-institutional phase I trial combines everolimus with four-drug reinduction for pediatric ALL patients in first bone marrow relapse. Finally, a single-institution phase I/II trial investigating everolimus in combination with hyper-CVAD chemotherapy is underway for patients aged 10 years and older with relapsed/refractory ALL.

mTOR inhibitors such as rapamycin (sirolimus) are also effective in graft *versus* host disease (GVHD). The recently completed phase III COG trial, ASCT0431, investigated whether the addition of sirolimus to GVHD prophylaxis in children with ALL would decrease relapse rates as well as acute GVHD (aGVHD). Results showed that although sirolimus decreased aGVHD, survival was not improved [Pulsipher *et al.* 2014].

JAK/STAT

Hyperactive signaling of the Janus-associated kinase (JAK)/signal transducer and activator of transcription (STAT) pathway has been documented in several types of high-risk leukemia. Activating mutations of JAK2 are well documented in AML [Daver and Cortes, 2012]. Aberrant activation of JAK signaling has also been described in Philadelphia chromosome-like (Ph-like) ALL, a recently defined subtype of ALL with gene expression patterns similar to Ph+ cases but lacking the BCR-ABL fusion [Mullighan *et al.* 2009]. Xenograft models of eight cases of Ph-like ALL demonstrated decreased leukemic burden when treated with a selective JAK1/2 inhibitor, ruxolitinib, and six of these xenograft models harbored either a JAK2 mutation or CRLF2 rearrangement [Maude *et al.* 2012]. The remaining two Ph-like ALL patient samples contained some other activating signature of hyperactive JAK/STAT signaling, despite lacking a point mutation. This suggests that a JAK2 activation footprint may be more significant than the presence of a mutation, in terms of predicting response to JAK2 inhibition. Interestingly, the mTOR pathway is also often aberrantly activated in Ph-like pre-B ALL patients, and single agent rapamycin demonstrated activity in all eight of these xenograft models [Maude *et al.* 2012]. Combining mTOR inhibitors with JAK2 inhibition, or combining JAK2 inhibition with cytotoxic chemotherapy, has not yet been studied in pediatric leukemia. The phase I COG study ADVL1011 recently investigated the safety and dosing of single-agent ruxolitinib in children with relapsed/refractory hematologic malignancies and solid tumors, and results are pending.

Furthermore, a detailed genomic analysis of 154 patients identified as Ph-like ALL by gene expression profiling was recently reported [Roberts *et al.* 2014]. Greater than 90% of patients in this cohort were found to have a kinase-activating lesion. Rearrangements involving ABL1, ABL2, and JAK2 were among the most common alterations, and fusion protein expression caused cell proliferation and activated STAT5 signaling. Importantly, Ph-like leukemia cells with fusions involving ABL 1/2 were sensitive to dasatinib *in vitro*, and cells with JAK2 rearrangements were sensitive to ruxolitinib. Trials are needed to determine whether identifying Ph-like patients and incorporating targeted TKIs into therapy will improve outcomes in this patient population.

JAK2 inhibitors were also identified in a high-throughput screen of kinase inhibitors as potential therapeutic agents in resistant FLT3-mutant AML [Weisberg *et al.* 2012]. The combination of JAK inhibitors with the FLT3 inhibitor PKC412 in AML cell lines demonstrated synergistic cytotoxicity and inhibition of downstream pathway signaling, specifically overcoming resistance to PKC412 in the presence of stroma [Weisberg *et al.* 2012]. An adult phase III study is investigating pacritinib, a dual JAK2/FLT3 inhibitor, in myelofibrosis [ClinicalTrials.gov identifier: NCT02055781].

Proteasome inhibitors

Proteasome inhibitors impair tumor growth through a variety of mechanisms [Rajkumar *et al.* 2005] and inactivate nuclear factor kB (NF-kB) by blocking the degradation of IkBa, a negative regulator of NF-kB. NF-kB is a transcriptional activator with anti-apoptotic properties, and is thought to be a key survival factor in various malignancies [Wang *et al.* 1996]. NF-kB is aberrantly constitutively activated in primary AML samples, specifically in the quiescent $CD34^+/CD38^-$ LSC compartment [Guzman *et al.* 2001]. NF-kB activity is also increased in leukemia cells after treatment with chemotherapy [Maestre *et al.* 2001]. Treatment of AML samples with a proteasome inhibitor led to down-regulation of NF-kB and its downstream targets, and induced apoptosis in $CD34^+$ leukemia cells, but not in normal $CD34^+$ bone marrow [Guzman *et al.* 2001]. Combining a proteasome inhibitor with idarubicin induced a rapid apoptotic response in primary AML samples *in vitro*, and effectively ablated the ability of the LSC population to engraft in a xenograft model, though normal hematopoietic cells were viable and able to engraft [Guzman *et al.* 2002]. This data led to the clinical investigation of a proteasome inhibitor, bortezomib, hypothesizing that selective targeting of the LSC population would result in more effective and durable responses in combination with standard treatment.

Bortezomib

Although bortezomib treatment inhibits NF-kB activity, it has little antileukemia activity as a single agent [Horton *et al.* 2007]. Adult trials have demonstrated that bortezomib may be safely combined with AML chemotherapy, although there have been rare cases of acute respiratory distress syndrome (ARDS) in combination with high-dose cytarabine [Attar *et al.* 2008]. The phase II COG protocol AAML07P1 combined bortezomib with standard AML chemotherapy and though results are not yet published, no children developed ARDS. Thus, while the available data on bortezomib in pediatric AML are limited, *in vitro* and adult data demonstrate that bortezomib may be combined safely with AML chemotherapy and may augment the efficacy of standard AML therapy. Phase III COG trial AAML1031 is evaluating bortezomib in a randomized fashion in newly diagnosed pediatric AML, in combination with standard chemotherapy.

A phase I TACL trial determined that bortezomib is well tolerated in combination with four-drug reinduction chemotherapy in relapsed/refractory pediatric ALL [Messinger *et al.* 2010]. The expanded phase II portion of this trial evaluated 22 relapsed ALL pediatric patients and demonstrated a 73% overall response rate, including 14 CRs and 2 CRis [Messinger *et al.* 2012]. The recently completed phase II COG study AALL07P1 combined bortezomib with reinduction chemotherapy for relapsed ALL. End of induction CR was achieved in 42 of 61 (68%) of pre-B ALL patients, as well as 11 of 17 (65%) T-cell ALL patients (unpublished data, Horton, COG open meeting, 2013), prompting the planned incorporation of bortezomib in a randomized fashion in the next phase III COG study for newly diagnosed T-cell ALL, AALL1231.

Carfilzomib

Carfilzomib is another proteasome inhibitor that is structurally and mechanistically distinct from bortezomib, demonstrating less reactivity against non-proteosomal proteases [Arastu-Kapur *et al.* 2011] and achieving higher levels of proteasome inhibition than bortezomib in preclinical models [Yang *et al.* 2011]. In early phase trials of adults with multiple myeloma, carfilzomib showed good tolerability both alone and in combination with chemotherapy, and clinical responses were achieved in both bortezomib-naïve and bortezomib pretreated patients [Siegel *et al.* 2012; Wang *et al.* 2013]. Carfilzomib induced apoptosis in a variety of pediatric tumor cell lines, and synergized with etoposide and cyclophosphamide [Jayanthan *et al.* 2013; Ruan *et al.* 2013]. Based on this encouraging preclinical data, a phase I Pediatric Oncology Experimental Therapeutics Investigator's Consortium (POETIC) study is planned, evaluating the tolerability of carfilzomib with cyclophosphamide and etoposide in relapsed/refractory pediatric leukemia and solid tumors. A TACL phase I/II study is also planned for children with relapsed/refractory leukemia, investigating carfilzomib in combination with UKALLR3 reinduction chemotherapy.

Epigenetic targeting

Epigenetic modifications, including methylation of CpG islands in gene promoter regions and chromatin modifications by histone acetylation, play a critical role in gene expression. Transcriptional silencing of tumor suppressor genes can lead to malignant transformation in many cancers, including leukemia [Herman *et al.* 1997; Seedhouse *et al.* 2003]. These modifications can be targeted by DNA methyltransferase (DNMT) inhibition or histone deacetylase (HDAC) inhibition in an attempt to reverse the epigenetic silencing of crucial regulatory genes and modify the malignant phenotype [Gore, 2005].

DNMT

The two azanucleosides, 5-azacytidine (azacitidine) and 5-aza-2′-deoxycytidine (decitabine), are cytosine analogs that incorporate into nucleic acids, thereby affecting multiple molecular pathways. After incorporation into DNA, both drugs covalently 'trap' DNMTs, which are then degraded [Santi *et al.* 1984]. There is *in vitro* evidence that these drugs are effective in inducing DNA demethylation and epigenetic gene reactivation [Stresemann *et al.* 2006], although differential demethylating effects on various human AML cell lines is seen [Stresemann *et al.* 2008].

Both azacitidine and decitabine are effective in the treatment of adult patients with MDS [Silverman *et al.* 2002; Kantarjian *et al.* 2006], and have been FDA-approved for this disease. Decitabine has also been approved in Europe for the treatment of adult AML. Combining DNMT inhibitors with HDAC inhibitors is being studied extensively in adult MDS and AML, with promising initial results [Garcia-Manero *et al.* 2006;

Gore *et al.* 2006; Soriano *et al.* 2007]. Some studies suggest that despite good clinical responses, no correlation is seen between response and methylation or gene expression patterns [Fandy *et al.* 2009]. However, another study compared gene expression and methylation patterns of paired diagnostic-relapsed ALL samples, and demonstrated a relapse-specific signature associated with chemoresistance [Bhatla *et al.* 2012]. Treating primary relapsed samples or leukemia cell lines *in vitro* with the HDAC inhibitor vorinostat alone or in combination with decitabine restored expression of genes that were preferentially silenced at relapse, and seemed to ‘reprogram’ the abnormal relapsed gene expression profile. Furthermore, these drugs demonstrate synergistic cytotoxicity when given prior to chemotherapy [Bhatla *et al.* 2012]. A recently completed phase I TACL trial combined azacitidine with chemotherapy for relapsed/refractory pediatric ALL or AML, and an ongoing phase I/II TACL trial is evaluating the efficacy of epigenetic therapy with decitabine and vorinostat followed by reinduction chemotherapy in relapsed/refractory pediatric ALL.

HDAC

HDACs remove acetyl groups from lysine residues on histones, causing decreased accessibility of chromatin and thus, transcriptional repression and epigenetic silencing [Lucas *et al.* 2010]. *In vitro* experiments demonstrate efficacy of HDAC inhibitors in both AML and ALL. One investigation demonstrated synergy of vorinostat and methotrexate in ALL cell lines by increasing the expression of the synthetase that metabolizes methotrexate to its active form, leading to increased cytotoxicity [Leclerc *et al.* 2010]. Another study demonstrated restoration of BH3-only BCL2 family member (BIM) expression after vorinostat treatment in glucocorticoid-resistant ALL xenografts, resensitizing this disease to glucocorticoids [Bachmann *et al.* 2010]. In an AML cell line, treatment with vorinostat resulted in increased expression of p21, leading to p53-independent cell cycle arrest [Vrana *et al.* 1999]. HDAC inhibitors have also been shown to abolish the fusion proteins AML1-ETO and PML-RARa in AML [Kramer *et al.* 2008]. Stumpel and colleagues demonstrated that treatment with HDAC inhibitors induced cytotoxicity in t(4;11) MLL-r primary infant ALL cells, and HDAC inhibitor treatment was shown to neutralize the MLL-AF4 fusion protein [Stumpel *et al.* 2012]. These data and others led to the investigation of HDAC inhibitors in acute leukemia.

A phase I adult trial with vorinostat monotherapy demonstrated some antileukemia effect and good tolerability [Garcia-Manero *et al.* 2008]. Subsequently, a phase I pediatric trial with vorinostat in solid tumors and leukemia also demonstrated tolerability, although patients with refractory leukemia required a lower dose [Fouladi *et al.* 2010]. As stated above, vorinostat is currently being investigated in a phase II pediatric clinical trial in combination with decitabine in relapsed ALL, in an attempt to ‘reprogram’ the gene expression signature and restore sensitivity to chemotherapy. In addition, a phase I TACL trial recently opened and is investigating the tolerability of the newer HDAC inhibitor, panobinostat, in refractory pediatric hematological malignancies.

DOT1L

Approximately 60–80% of infant leukemia harbor rearrangements of the MLL gene (MLL-r) [Ayton and Cleary, 2001] and have a poor overall prognosis with standard chemotherapy approaches. Similar gene expression patterns have been described in MLL-r leukemia despite the variety of possible fusion proteins [Tsutsumi *et al.* 2003], including upregulation of homeobox (Hox) gene expression [Krivtsov *et al.* 2006]. It has been demonstrated that various MLL-fusion proteins form a complex with Disruptor of telomeric signaling-1 (DOT1L) [Zhang *et al.* 2006; Buttner *et al.* 2010]. DOT1L is the sole methyltransferase that methylates lysine 79 of histone 3 (H3K79), causing transcriptional activation [Lacoste *et al.* 2002]. The interaction of MLL-fusion proteins with DOT1L can lead to the mistargeting of DOT1L and subsequent methylation of H3K79 at inappropriate gene promoter sites, likely playing an important role in leukemogenic transformation [Okada *et al.* 2005; Mueller *et al.* 2007; Krivtsov *et al.* 2008]. For example, hypermethylation of H3K79 is seen at the promoter of HoxA9 in MLL-r leukemia [Krivtsov *et al.* 2008; Yokoyama *et al.* 2010].

In vivo and *in vitro* experiments have demonstrated that H3K79 methylation by DOT1L is in fact required for both initiation and maintenance of MLL-AF9 induced leukemogenesis [Chang *et al.* 2010; Bernt *et al.* 2011; Nguyen *et al.* 2011].

A potent small molecule inhibitor of DOT1L, EPZ004777, selectively killed MLL-r cells while having little effect on non-MLL translocated cells, and led to increased survival in an MLL-AF9 xenograft model [Daigle *et al.* 2011]. Similar results were then demonstrated in MLL-AF10 and MLL-AF6 cells [Chen *et al.* 2013; Deshpande *et al.* 2013]. Furthermore, treatment with EPZ004777 led to decreased expression of HoxA9 and Meis1 in all MLL-rearranged leukemia cells. These data provide strong rationale for targeting DOT1L in MLL-r leukemia. A phase I/II trial of DOT1L inhibitor EPZ-5676 is currently underway for adults with advanced MLL-r hematological malignancies [ClinicalTrials.gov identifier: NCT01684150], and a phase I multi-institutional trial of EPZ-5676 recently opened for pediatric patients with relapsed/refractory MLL-r leukemia.

Antibody-based immunotherapy

CD33

CD33 cell surface antigen is expressed in 88% of childhood AML blasts [Creutzig *et al.* 1995]. CD33 is also present on normal maturing hematopoietic progenitor cells, but is not present on hematopoietic stem cells or other normal tissue, making it an attractive target [Sievers *et al.* 2001]. Gemtuzumab (CMA-676 or GO) is an anti-CD33 antibody conjugated to calicheamicin, a potent antitumor antibiotic that cleaves double-stranded DNA at specific sequences, leading to apoptosis [Zein *et al.* 1988; Hinman *et al.* 1993; Sievers *et al.* 1999]. COG phase III study AAML0531 incorporated GO in a randomized fashion in combination with standard chemotherapy in *de novo* AML. Results showed a modest improvement in 3-year EFS in the GO arm (53% *versus* 47%, $p = 0.05$) and a trend toward improved OS [Aplenc *et al.* 2013]. However, GO was withdrawn from the US market in 2010 after adults receiving GO in induction therapy experienced increased mortality, despite recently published studies of GO in children [Burnett *et al.* 2011; Cooper *et al.* 2012; Hasle *et al.* 2012] and adults [Castaigne *et al.* 2012] showing that a dose of 3 mg/m^2 has been well tolerated. Furthermore, use of GO as consolidation therapy after HSCT in AML patients [Roman *et al.* 2005], or as part of a conditioning regimen for HSCT [Satwani *et al.* 2012] in early phase studies demonstrated safety in both settings. Reports also demonstrate reduction of MRD status with GO in otherwise refractory pediatric AML patients, thereby optimizing disease status prior to HSCT [Rubnitz *et al.* 2010; O'Hear *et al.* 2013]. These reports provide good rationale to consider 'resurrecting' GO [Ravandi *et al.* 2012].

CD22

CD22 is expressed in 96% of pre-B ALL patients on at least 90% of blasts [Gudowius *et al.* 2006]. Epratuzumab is a humanized monoclonal anti-CD22 antibody, and cells demonstrate rapid intracellular localization of the antibody complex after binding [Carnahan *et al.* 2003]. COG phase I/II study ADVL04P2 combined epratuzumab with reinduction chemotherapy in children with CD22$^+$ relapsed ALL, and response was assessed after block one. In the phase II portion of the study, 65% of 98 evaluable patients achieved a CR, and 46% of these were MRD negative [Raetz *et al.* 2011]. This was significantly higher than the 25% of CR patients achieving negative MRD with chemotherapy alone on predecessor study AALL01P2 ($p = 0.001$). The upcoming European phase III IntReALL study will randomize pediatric ALL patients in standard risk first relapse to receive epratuzumab.

Since CD22 is internalized upon binding of monoclonal antibody, it is attractive for targeted delivery of immunotoxin [Press *et al.* 1989]. Inotuzumab ozogamicin (CMC-544 or IO) is an anti-CD22 humanized monoclonal antibody conjugated to calicheamicin, similar to anti-CD33 GO [DiJoseph *et al.* 2004]. IO was originally developed for use in CD22-expressing non-Hodgkin lymphoma (NHL), and two early phase trials of IO in adults with refractory NHL showed promising clinical responses as a single agent [Advani *et al.* 2010] and in combination with rituximab [Fayad *et al.* 2013]. Preclinical evidence with pre-B ALL cell lines and mouse models demonstrated that IO has good cytotoxic effect against CD22+ lymphoblasts *in vitro*, and abrogates xenograft tumors and disseminated leukemia *in vivo* [Dijoseph *et al.* 2007]. IO demonstrated clinical activity in a phase II study of adults and children with relapsed/refractory CD22+ pre-B ALL at dosing of 1.3–1.8 mg/m^2 every 3–4 weeks [Kantarjian *et al.* 2012]. Of 49 patients, 28 (57%) achieved CR, CRi or complete response with incomplete platelet recovery (CRp; platelets $<100 \times 10^9$/L). An additional 41 patients were enrolled at 1.8 mg/m^2 divided into a weekly dosing regimen (0.8, 0.5, and 0.5 mg/m^2/week) and had a similar overall response rate of 59%

[Kantarjian *et al.* 2013]. The most common toxicities were grade 1–2 fever, hypotension, elevated transaminases and hyperbilirubinemia. A retrospective review of the five pediatric patients on this trial demonstrated good tolerability and activity at both dosing schedules [Rytting *et al.* 2014]. Three of five patients achieved a CR or CRp and went to HSCT, including one patient who had achieved an MRD negative status after two cycles of the weekly dosing schedule. Current trials are investigating IO in adults with ALL in relapsed disease (phase III trial [ClinicalTrials.gov identifier: NCT01564784]), in the elderly population (phase I/II trial [ClinicalTrials.gov identifier: NCT01371630]), and prior to allogeneic transplant (phase I/II trial [ClinicalTrials.gov identifier: NCT01664910]). There are no open pediatric trials investigating IO at the time of this review.

Moxetumomab pasudotox (MP, previously CA-8015 or HA22) is a second-generation anti-CD22 immunotoxin, composed of the variable fragment of the antibody (Fv) conjugated to a protein derivative (PE38) of *Pseudomonas* exotoxin A [Pastan *et al.* 2006]. BL22, the first-generation predecessor of moxetumomab, demonstrated cytotoxicity against malignant CD22$^+$ cells *in vitro* [Kreitman *et al.* 2000] and clinical activity in a phase I trial of pediatric ALL patients [Wayne *et al.* 2010]. Likewise, moxetumomab demonstrated good *in vitro* activity against relapsed, steroid-resistant, and *de novo* pediatric pre-B ALL samples [Mussai *et al.* 2010]. Immunotoxin-resistant ALL cells were found to be heavily hypermethylated at the DPH4 promoter, rendering these cells refractory to moxetumomab, and suggesting a role for concurrent demethylating agents [Wei *et al.* 2012]. A phase I study of moxetumomab in CD22$^+$ pediatric malignancies is ongoing, and preliminary results report 4 CRs in 17 evaluable patients (24%), 1 partial response (6%) and 7 patients (41%) with >50% reduction in peripheral blood blasts or improvement in blood counts [Wayne *et al.* 2011]. Toxicities were generally mild and reversible, although capillary leak syndrome was dose limiting in several patients. Fourteen percent of patients developed neutralizing antibodies, necessitating further study of the potential challenges of this drug. A phase II multicenter study of moxetumomab recently opened in August 2014 for relapsed/refractory pre-B ALL or B-cell lymphoblastic lymphoma.

CD19

Blinatumomab is a CD19/CD3 bispecific T-cell engaging (BiTE) antibody that binds to CD3$^+$ T-cells and colocalizes them with CD19$^+$ B-cells, thereby activating the T-cells and inducing perforin-mediated death of the targeted B-cells [Loffler *et al.* 2000]. After promising results in a phase I trial of adults with NHL [Bargou *et al.* 2008], blinatumomab was moved in a phase II study in adult pre-B ALL. This study yielded striking results; of 20 evaluable patients with detectable MRD by polymerase chain reaction (PCR), 16 (12 primary refractory and 4 relapsed) achieved an MRD negative remission. Eight of these 16 patients received a HSCT and remained in remission post-transplant. Common adverse events were pyrexia, chills and lymphopenia with hypogammaglobulinemia [Topp *et al.* 2011]. At a median follow-up of 33 months, relapse-free survival in these patients was 61% overall, with 6 relapses reported [Topp *et al.* 2012]. Of 4 earlier relapses (at 3–7 months), two had CD19-negative marrow disease, one had an isolated CNS relapse, and one had a testicular relapse. Interestingly, the CNS and testes are relatively T-cell deplete compartments. The two later relapses (at 19 and 31 months) were both CD19$^+$ [Topp *et al.* 2012]. Importantly, four responders received no subsequent therapy yet remain in remission, suggesting that as a single agent, blinatumomab can induce a durable remission in MRD-positive disease. Remarkably, the effector memory T-cell subset was amplified in all patients, which could partially explain the duration of response in these patients, though amplification occurred in non-responders as well [Klinger *et al.* 2012]. The US Intergroup ECOG is currently investigating blinatumomab in upfront adult pre-B ALL in a phase III clinical trial [ClinicalTrials.gov identifier: NCT02003222].

In children, Handgretinger and colleagues evaluated blinatumomab monotherapy in a small case series of heavily pretreated, post-HSCT pediatric ALL patients. All three of three children achieved negative MRD with tolerable and similar toxicities. Two of three ultimately relapsed, one after a second transplant, and the other after four courses of blinatumomab. The third patient received a second transplant and remained in a CR at 23 months [Handgretinger *et al.* 2011]. A multicenter phase I trial in 34 children with relapsed/refractory pre-B ALL administered blinatumomab as a continuous 4-week infusion, and established the maximum tolerated dose (MTD) as 15 μg/m²/day [Zugmaier *et al.* 2013]. The dose-limiting toxicity was cytokine-release syndrome. Across all dose levels, the overall response was 41% [Zugmaier *et al.* 2013]. A randomized COG phase III study for pre-B ALL children in first relapse is scheduled to open in late 2014, combining blinatumomab with UKALLR3 reinduction chemotherapy.

Cellular-based immunotherapy

Chimeric antigen receptor-modified T-cells (CAR T-cells) with CD19 specificity are generating excitement as a novel therapy for B-cell malignancies. CAR T cells are patient-derived T-cells, transduced to express a chimeric antigen receptor, which includes an anti-CD19 antibody fragment fused to a T-cell intracellular signaling domain [Barrett *et al.* 2014]. Second-generation CAR T cells also encode for a costimulatory domain, such as CD28 or members of the tumor necrosis factor receptor family such as CD27, CD137 (4-1BB) and CD134 (OX40). The costimulatory domains activate the CAR T-cells, allowing for targeting and lysis of $CD19^+$ cells. Clinical trials with second-generation CD19 CAR T-cells in adult chronic lymphocytic leukemia (CLL) demonstrated massive T-cell expansion *in vivo*, lysis of $CD19^+$ tumor cells, and aplasia of normal $CD19^+$ B cells [Kalos *et al.* 2011]. Importantly, these relapsed and refractory CLL patients demonstrated durable remissions after infusions of CD19 CAR T-cells.

Several institutions in the United States have open pediatric trials of CD19 CAR T-cells in pre-B cell ALL. The first two pediatric patients treated at Children's Hospital of Philadelphia (CHOP) with refractory/relapsed pre-B ALL achieved a CR within a month of CAR T-cell infusion [Grupp *et al.* 2013]. In both patients, dramatic expansion of CAR T-cells was documented in the peripheral blood, peaking around day 10. Surprisingly, CAR T-cells were detected in the CNS in both patients. Toxicity related to a cytokine-release syndrome after CAR T-cell infusions is significant, and in severe cases can be reminiscent of macrophage-activation syndrome. However, anticytokine therapy with the anti-IL6 monoclonal antibody, tocilizumab, can rapidly induce clinical improvement in this setting [Grupp *et al.* 2013]. As expected, complete B-cell aplasia occurs, and the duration of this effect is undefined and must be supported with intravenous immunoglobulin (IVIG) administration. Of the original two pediatric patients, one remains in remission without any subsequent disease-directed therapy. The other patient relapsed in 2 months with a CD19-negative clone, signifying the strong selective pressure of this therapy.

At the American Society of Hematology (ASH) 2013 annual meeting, the CHOP group presented their updated outcomes on this trial, having now enrolled 16 pediatric and 4 adult patients with $CD19^+$ leukemia. A total of 14 of 17 evaluable patients (82%) achieved a CR at 1 month. Three of these 14 CR patients subsequently relapsed. Aside from the patient mentioned above, the other two relapsed patients did not have a CD19 negative clone. All patients have demonstrated some sort of cytokine release syndrome corresponding with the peak T-cell expansion *in vivo* [Frey *et al.* 2013]. Seven of 20 required treatment for respiratory or hemodynamic instability, and all improved rapidly after tocilizumab given with or without steroids. In all patients with durable responses, CAR T-cells were detectable by flow cytometry for a range of 1–15 months. Given these encouraging results, multicenter pediatric trials are now being planned to investigate CD19 CAR T-cells in a phase II setting.

Finally, a phase I clinical trial of CD22 CAR T-cells for pediatric patients with relapsed/refractory ALL is currently in development, based on promising preclinical data [Haso *et al.* 2013].

Conclusion

There is compelling rationale for the ongoing discovery and implementation of novel and targeted agents in pediatric leukemia. Future goals of treatment for pediatric ALL and AML, as well as other forms of cancer, are to personalize treatment to the specific molecular aberrancies driving each individual's disease, while decreasing the intensity and toxicities of conventional chemotherapy regimens. Pediatric leukemia is a disease prototype for this area of translational and clinical research, given the relatively smaller number of cumulative 'hits' necessary for malignant transformation. However, there are significant challenges to the development of novel agents. The process of drug development, including enrolling adequate numbers of patients to complete clinical trials, will be particularly challenging when only specific subgroups of leukemia patients are appropriate to study for each new agent. As mentioned previously, incorporating a new agent in the safest and most effective manner into existing chemotherapy regimens is complex, and this complexity will undoubtedly increase as combinations of targeted agents are proposed; particularly since another challenge is the development of resistance that occurs upon applying strong selective pressure with targeted agents. Finally, newer targeted agents must be effective in eradicating not only the bulk leukemia population, but also the elusive and often quiescent leukemic stem cell (LSC) population, in order to effectively prevent relapse and improve long-term survival. Despite these challenges, the ongoing discovery of oncogenic lesions in pediatric leukemia and the exciting parallel development of diverse novel therapeutic agents provide true promise for improving outcomes in high-risk and relapsed pediatric acute leukemia.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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