Novel therapies for children with acute myeloid leukaemia

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Abstract

Significant improvements in survival for children with acute myeloid leukaemia (AML) have been made over the past three decades, with overall survival rates now approximately 60-70%. However, these gains can be largely attributed to more intensive use of conventional cytotoxics made possible by advances in supportive care, and although over 90% of children achieve remission with frontline therapy, approximately one third in current protocols relapse. Furthermore, late effects of therapy cause significant morbidity for many survivors. Novel therapies are therefore desperately needed. Early phase paediatric trials of several new agents such as clofarabine, sorafenib and gemtuzumab ozogamicin have shown encouraging results in recent years. Due to the relatively low incidence of AML in childhood, the success of paediatric early-phase clinical trials is largely dependent upon collaborative clinical trial design by international cooperative study groups. Successfully incorporating novel therapies into frontline therapy remains a challenge, but the potential for significant improvement in the duration and quality of survival for children with AML is high.

Keywords

Acute myeloid leukaemia, AML, children, novel therapeutics, inhibitors, chemotherapy
Introduction

Acute myeloid leukemia (AML) is a heterogeneous class of leukaemias with an age-related incidence.\(^1\) AML is relatively rare in children, accounting for 15-20% of paediatric leukaemias, but causes a disproportionate number of childhood cancer deaths. For children less than 15 years of age overall survival (OS) rates are now approximately 60-70\%.\(^2\)-\(^5\)

Although there has been a steady and gradual improvement in survival for younger adults, it is clear that progress has plateaued for children, with survival rates being substantially inferior to those being achieved for acute lymphoblastic leukaemia (ALL), where OS exceeds 80\%.\(^4\),\(^6\)

Conventional AML therapy is essentially the same for adults and children and based on intensive use of cytarabine or other nucleoside analogs and anthracyclines. Etoposide is frequently used in paediatric induction regimens, although there is no good evidence it is of benefit in adult studies and when combined with cytarabine and daunorubicin in children, etoposide provided no advantage compared to thioguanine with cytarabine and daunorubicin.\(^2\),\(^7\) Although chemotherapy will induce complete remission (CR) in approximately 90% of children, approximately one third relapse.\(^4\),\(^5\) Relapsed AML is associated with high morbidity and mortality, with allogeneic haematopoietic stem cell transplantation (HSCT) generally regarded as the most effective anti-leukaemic therapy available.\(^6\) Most re-induction regimens, at least in children, involve high-dose cytarabine in combination with other agents such as anthracyclines. Addition of liposomal daunorubicin to the commonly utilized FLAG regimen (fludarabine, high-dose cytarabine and G-CSF) has been shown to improve the early response rates to chemotherapy from 58% to 68% (\(P = 0.047\)).\(^8\) Not surprisingly, the likelihood of achieving a second CR decreases dramatically...
with each failed therapeutic attempt, with only one third of children with relapsed AML becoming long-term survivors.

Although the past 30 years has seen major improvements in overall survival for childhood AML, additional gains are unlikely to be achieved by simply intensifying conventional cytotoxic chemotherapy further. Chemotherapy such as etoposide and anthracyclines is limited not only by acute toxicity, but also late effects such as increased risks of secondary malignancy and cardiotoxicity. Such late effects are of particular concern in paediatrics since treatment occurs during periods of growth and development, and the duration of survivorship is much greater than adults. Less toxic and more effective therapies for AML in children, and adults, are therefore urgently needed. The ever-expanding array of molecular abnormalities associated with AML promises to further refine current risk-stratified treatment and facilitate individualised therapy with novel therapeutics specifically targeting these leukaemogenic abnormalities. Such an approach should improve outcomes for those children who have historically done poorly and, potentially, permit de-intensification of therapy for those children with low-risk disease, as has been achieved with paediatric ALL.

Several early-phase clinical trials of novel therapies have been completed over the past decade and an encouraging number of studies are in progress (Table I). A major limitation for progress in childhood AML, however, is that paediatric studies with novel agents usually don’t commence until preliminary toxicity and efficacy profiles have been established in adults. Whilst this may be justifiable from a patient-safety perspective, it slows the process of paediatric drug development. Furthermore, it is increasingly recognized that AML biology is at least partially different in children compared to adults, so extrapolating anticipated efficacy from adult studies to children can be challenging. For example, ‘epigenetic’ mutations of
DNMT3A, IDH1/2, TET2 and NPM1 all occur much less frequently in children than in adults.\textsuperscript{12-16}

Novel classes of therapeutics investigated in childhood AML in recent years include purine nucleoside analogs, small molecule kinase inhibitors and immunomodulatory therapies. Here we review the results of these trials and highlight some of the more promising agents emerging from adult trials.

**Novel purine nucleoside analogs**

The cytidine analog and DNA polymerase inhibitor cytarabine forms the backbone of conventional AML therapy in both children and adults. It is not surprising that novel cytarabine derivatives have delivered some promising results. Cladribine and fludarabine were the first two such derivatives developed. Unlike cytarabine, they inhibit both DNA polymerase and ribonucleotide reductase, thereby depleting deoxynucleotide pools.\textsuperscript{17} Cladribine has shown encouraging results in combination with cytarabine\textsuperscript{18}, topotecan\textsuperscript{19} and idarubicin.\textsuperscript{20} Interestingly, the FAB M5 subtype of AML appears more responsive to cladribine than other morphological subtypes.\textsuperscript{21} Fludarabine, in combination with cytarabine \(\pm\) an anthracycline, has become a widely used re-induction regimen for children with relapsed AML.\textsuperscript{8,9,22,23} The dose-limiting neurotoxicity of cladribine and fludarabine however, prompted the development of clofarabine. After efficacy was demonstrated for relapsed/refractory ALL, in December 2004 clofarabine became the first anti-leukaemic drug approved for use in childhood prior to adult use approval.\textsuperscript{17} The efficacy and potential role of clofarabine in paediatric AML is discussed in detail below.
**Clofarabine**

Clofarabine is a structural hybrid of cladribine and fludarabine, designed to have improved efficacy, through reduced deamination by adenosine deaminase and improved stability, whilst avoiding the formation of toxic metabolites such as 2-F adenine caused by glycosidic bond cleavage of fludarabine. Clofarabine is converted intracellularly to clofarabine monophosphate by deoxycytidine kinase (dCK), then to clofarabine triphosphate by additional kinases. The triphosphate form of clofarabine impairs DNA synthesis and repair via inhibition of both ribonucleotide reductase and DNA polymerase and induces apoptosis via mitochondrial pathways. As a potent ribonucleotide reductase inhibitor, clofarabine increases dCK activity, thus potentiating its own activation and theoretically that of other drugs such as cytarabine. The maximum tolerated dose (MTD) as a single-agent is 52 mg/m²/day for 5 days in children and 40mg/m²/day for 5 days in adults with hematologic malignancies, with reversible hepatotoxicity and skin rash being the main non-haematological dose limiting toxicities (DLTs). Although the liver and skin toxicities are reversible when clofarabine is given as a single agent, it should be noted that a potential interaction with intrathecal methotrexate exists, with at least one case report describing fatal skin and hepatotoxicity in a child with a CNS-relapse of pre-B ALL given clofarabine 52 mg/m²/day for 5 days, together with triple intrathecal therapy (hydrocortisone, methotrexate and cytarabine).

A phase II study of clofarabine as a single agent in children with relapsed/refractory AML had an overall response rate (ORR) of 26%, comprising one CR with incomplete platelet recovery (CRp) and 10 partial responses (PR). Although most responses were partial, the trial population was heavily pretreated, with patients having received a median of two prior treatment regimens (range one to five). Furthermore, six of 28 (21%) patients refractory to...
prior therapy responded and approximately one third of clofarabine treated patients proceeded to HSCT. Subsequent interpretation of the HSCT data is problematic however, considering that most patients went to HSCT in PR, rather than CR. The toxicity profile was reported as being expected for the patient population, with frequent (≥ 15%) grade 3 adverse events being febrile neutropaenia, catheter-related infection, epistaxis, hypotension, nausea, fever, elevated transaminases and hypokalaemia.

Clofarabine has also been trialed in combination with conventional and targeted therapeutic agents. In a phase I study of the multi-kinase/FLT3 inhibitor sorafenib in combination with clofarabine and cytarabine, responses were seen in both FLT3-wild type (-WT) and FLT3-ITD+ patients. This trial is discussed further below. A phase I trial of clofarabine in combination with etoposide and cyclophosphamide for children with relapsed and refractory acute leukemias resulted in an ORR of 55% (9/20 CR + 2/20 CRp) for ALL patients and 100% (1/5 CR + 4/5 CRp) for AML patients. When given for five consecutive days during induction and four consecutive days in consolidation for up to eight cycles (median time between cycles 34 days), the recommended phase II doses (RP2D) of clofarabine, cyclophosphamide and etoposide were 40, 440 and 100 mg/m² respectively. The toxicity profile was similar to that seen in the monotherapy phase II study of clofarabine.

A phase I study of clofarabine in combination with liposomal daunorubicin for children with relapsed/refractory AML was recently completed. Patients received clofarabine on days 1-5 (30 mg/m², escalated to 40 mg/m²), and liposomal daunorubicin (60 mg/m²) days 1, 3 and 5. Nine patients were enrolled, with the most common severe adverse event being febrile neutropaenia, but no DLTs observed. One third of patients achieved CR and proceeded to HSCT. The 40 mg/m² clofarabine cohort is being extended to accrue more efficacy data.
There is intense ongoing interest in clofarabine for AML in adults and children, with no fewer than 35 open studies of the drug in AML currently registered on ClinicalTrials.gov. A major challenge, however, remains in defining how best to use clofarabine in both upfront and second-line AML treatment regimens. Results of randomised studies in adults are expected in the near future, including the NCRI AML16 trial for adults over 60 years of age, which randomised newly-diagnosed patients to induction with either daunorubicin and cytarabine or daunorubicin and clofarabine (ISRCTN 11036523). The current NCRI AML17 trial for younger adults with AML is randomising poor risk patients (defined by a statistically-derived risk score based on presenting disease characteristics and remission status after course I chemotherapy), to either daunorubicin plus clofarabine or FLAG-Ida prior to HSCT (ISRCTN55675535). For childhood AML, a randomised phase II study sponsored by St Jude Children’s Research Hospital (SJCRH) is ongoing, comparing end-of-induction MRD for children treated with clofarabine and cytarabine to conventional ADE therapy (cytarabine, daunorubicin, etoposide; NCT00703820).

**FLT3 inhibition**

Internal tandem duplication (ITD) or tyrosine kinase domain (TKD) mutations of the fms-like tyrosine kinase 3 (FLT3) gene occur frequently in AML, resulting in constitutive FLT3 signaling and stimulation of leukaemic proliferation.\(^{30}\) The incidence of FLT3-ITD in AML increases with age, occurring in approximately 23% of adults and 12% of children overall with de novo AML, whilst the incidence of FLT3-TKD is relatively constant across all age groups at approximately 7%.\(^{31,32}\) Although FLT3-TKD mutations do not appear to impact on prognosis, the presence of FLT3-ITD has consistently been associated with inferior outcome, principally as a result of increased relapse rate.\(^{33}\) High FLT3-ITD/FLT3-WT allelic ratios
(AR) carry a particularly poor prognosis, with survival rates of less than 20% in both adults and children.\textsuperscript{31,34,35} Furthermore, \textit{ex vivo} studies have demonstrated increased dependence of high AR FLT3-ITD+ AML blasts to FLT3 signaling, consistent with the concept of “oncogene addiction”.\textsuperscript{36} The identification of secondary FLT3-TKD mutations in patients treated with FLT3 inhibitors is also evidence of clonal selection and cellular dependency on constitutive FLT3 signaling.\textsuperscript{37-39}

Small molecule inhibition of FLT3 kinase has therefore been vigorously pursued as a therapeutic strategy over the past decade and detailed critiques of each individual FLT3 inhibitor developed to date have recently been published.\textsuperscript{33,40} Initial “first generation” FLT3 inhibitors, such as CEP-701 (lestaurtinib) and PKC412 (midostaurin), were non-selective compounds already in early clinical development and subsequently identified as having potent anti-FLT3 activity. These compounds have undergone extensive preclinical and clinical evaluation with major phase III trials ongoing. So-called “second-generation” FLT3 inhibitors such as AC220 (quizartinib), designed with increased potency and selectivity for FLT3, are currently being evaluated in early-phase clinical trials.

Preclinical studies have demonstrated that sustained inhibition of FLT3 phosphorylation to less than 15% of baseline is required to achieve cytotoxicity.\textsuperscript{41,42} Furthermore, several pharmacokinetic (PK) and pharmacodynamic (PD) studies from clinical trials have consistently confirmed that clinical responses are unlikely in patients who do not achieve adequate FLT3 inhibition.\textsuperscript{41-45}

Finally, although newer more potent and selective FLT3 inhibitors such as AC220 are showing encouraging results in single-agent trials, it appears that FLT3 inhibitors are likely to
be of most benefit when used in combination with conventional chemotherapy.\textsuperscript{33} It is worth noting that myelosuppression from conventional chemotherapy itself has been shown to increase the production of FLT3 ligand (FL) which can decrease the efficacy of a number of FLT3 inhibitors, at least \textit{in vitro}.\textsuperscript{46} These observations highlight the need for effective PD biomarker assays to monitor how effectively FLT3 is being targeted. Assays such as the plasma inhibitory activity (PIA) assay have been used to demonstrate that effective FLT3 inhibition can still be achieved clinically however, despite elevations in FL (discussed in more detail below).\textsuperscript{47}

\textbf{Sorafenib}

The multi-kinase inhibitor, sorafenib, has potent activity against FLT3 and increased activity against \textit{FLT3-ITD\textsuperscript{+}} AML cells compared to \textit{FLT3-WT} cells.\textsuperscript{48} Although sorafenib was assessed by the Pediatric Preclinical Testing Program (PTPP), only one AML cell line was tested.\textsuperscript{49} In the PTPP screen, the \textit{FLT3-WT} cell line Kasumi-1 had an \textit{in vitro} IC\textsubscript{50} of 0.02 \(\mu\)M.\textsuperscript{49} Pre-clinical studies of sorafenib and another multi-kinase inhibitor, sunitinib, at SJCRH examined a broader panel of AML cell lines and demonstrated similar \textit{in vitro} sensitivity of Kasumi-1 (sorafenib IC\textsubscript{50} 0.04 \(\mu\)M), but markedly increased sensitivity of the \textit{FLT3-ITD\textsuperscript{+}} paediatric cell line MV4-11 (sorafenib IC\textsubscript{50} 0.002 \(\mu\)M). \textit{In vitro} activity of sorafenib against primary paediatric AML samples (4/6 \textit{FLT3-WT}, 2/6 unknown \textit{FLT3} status) was also demonstrated.\textsuperscript{50} Increased \textit{in vitro} sensitivity of \textit{FLT3}-mutated, primary paediatric AML samples has also been demonstrated for SU11657, a compound similar to sunitinib with anti-FLT3 activity.\textsuperscript{51} Unlike sorafenib, sunitinib is no longer being evaluated as a FLT3 inhibitor in AML due to poor tolerability at doses required to inhibit FLT3.\textsuperscript{33}
Several clinical trials of sorafenib in adult AML have been reported\textsuperscript{13}, with a recent phase I/II study in combination with cytarabine and idarubicin demonstrating overall CR rates of 93% (14/15, plus 1 with CRp) in FLT3-ITD\textsuperscript{+} patients and 66% (24/36 plus 3 with CRp) in FLT3-WT patients.\textsuperscript{52} In the phase II arm of the study, sorafenib was given at a dose of 400 mg twice daily for up to 28 days during induction and consolidation (both with concurrent cytarabine and idarubicin), then continued as maintenance therapy for up to one year.\textsuperscript{52} In contrast, a placebo-controlled, randomized phase II study of sorafenib combined with standard 7+3 induction chemotherapy and intermediate-dose cytarabine consolidation then continued for 1 year as maintenance in 197 older patients (> 60 years) failed to show any difference in CR rates, EFS, OS for FLT3-WT or FLT3-ITD\textsuperscript{+} patients, although the small number of FLT3-ITD\textsuperscript{+} patients (n = 28; 14.2%) may have limited the power of subset analysis. Despite being reportedly well tolerated, there was a trend towards slower leucocyte and platelet recovery in the sorafenib arm during the induction cycles.\textsuperscript{53}

Encouraging results from a paediatric phase I study of sorafenib in combination with cytarabine and clofarabine for children with relapsed/refractory acute leukaemia were recently published. Sorafenib was given as a single agent on days 1 to 7 and then concurrently with clofarabine (40 mg/m\textsuperscript{2}, or 20 mg/m\textsuperscript{2} if the patient had HSCT within 6 months or fungal infection within 1 month) and cytarabine (1 g/m\textsuperscript{2}) on days 8 to 12. If tolerated, sorafenib alone was continued until day 28. Repeated courses of sorafenib, clofarabine and cytarabine, maintenance therapy with single-agent sorafenib, or transplantation were administered according to clinical judgment.\textsuperscript{27} Eleven of the 12 patients (aged 6-17 years) had AML, including 3 who had previously undergone allogeneic HSCT (2 FLT3-ITD\textsuperscript{+}, 1 FLT3-WT). Of these 11 AML patients, 5 were FLT3-ITD\textsuperscript{+} and all achieved morphological CR on day 22 (3 CR + 2 CRp) in combination with cytarabine and
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clofarabine, including both patients who had previous allogeneic HSCT.\textsuperscript{27} Of the 6 \textit{FLT3-WT} AML patients, 3 achieved morphological CR and one a PR. Of note, the initial seven-day course of single-agent sorafenib reduced bone marrow blast percentages in 10 patients (median 66\%, range 9-95\%), including all \textit{FLT3-ITD}\textsuperscript{+} patients. Ten patients had PD studies performed (flow cytometric analysis of phosphorylated AKT, 4E-BP1 and S6RP), with decreases in percentage of positive cells and mean fluorescence intensity unrelated to \textit{FLT3-}
mutational status.\textsuperscript{27} Six patients proceeded to HSCT.\textsuperscript{27} The most common toxicity associated with sorafenib was skin toxicity, with all 12 patients experiencing some degree of hand-foot skin reaction and/or rash. Skin toxicity was dose-limiting at 200 mg/m\textsuperscript{2} but not 150 mg/m\textsuperscript{2}, which was the final recommended dose of sorafenib.\textsuperscript{27} The hand-foot skin (HFS) reaction to sorafenib is classically characterised by tender, scaling skin with erythematous halos on areas of pressure or skin creases which may blister and can progress to hyperkeratosis and impaired movement and function.\textsuperscript{54} Consensus guidelines for the management of sorafenib and sunitinib induced HFS reactions have been developed and include avoidance of skin trauma, liberal use of emollients and dose reduction/cessation of sorafenib.\textsuperscript{54} The high incidence of skin toxicity in this paediatric study compared to previous single agent adult trials was attributed the concurrent use of cytarabine and clofarabine, as well as the higher concentration of the active metabolite sorafenib N-oxide observed in the paediatric trial.\textsuperscript{27}

The current Children’s Oncology Group phase III trial (AAML1031, NCT01371981) is using a \textit{FLT3-ITD/FLT3-WT} allelic ratio >0.4 (high-AR) to non-randomly stratify children with \textit{FLT3-ITD}\textsuperscript{+} AML to receive sorafenib and HSCT in first remission, if an appropriate donor is available. Although the HSCT data for adults is inconclusive\textsuperscript{2}, matched related donor allogeneic HSCT has been demonstrated to be of benefit for \textit{FLT3-ITD}\textsuperscript{+} children with a high-AR.\textsuperscript{31} The dismal prognosis for children with high-AR \textit{FLT3-ITD}\textsuperscript{+} AML (3 year DFS/OS ≤
20% clearly warrants novel treatment approaches however, the number of children with high-AR disease transplanted on the CCG-2941 and CCG-2961 trials was small (total FLT3-ITD+ = 11; high-AR = 6), and all high-AR patients on the current COG AAML1031 trial will receive both sorafenib and allogeneic HSCT, so it may be difficult to determine the extent to which sorafenib further improves survival when compared to this historical control. 

Paediatric trials of other FLT3 inhibitors, including AC220, CEP-701 and PKC412, are currently in progress (Table I). An emerging issue with a number of FLT3 inhibitors, including midostaurin and the more selective compounds AC220 and sorafenib, is resistance mediated by secondary FLT3-TKD mutations. Given the biologically similar nature of FLT3-ITD+ AML in children and adults, similar resistance patterns could be expected although the relatively small number of children with FLT3-ITD+ AML may make this a rare occurrence. Finally, the benefit of “maintenance” FLT3 inhibition (e.g. post-HSCT) is yet to be proven, however it is plausible that such strategies with compounds like AC220 or sorafenib may promote acquired resistance, mediated by secondary FLT3-TKD mutations. 

Despite the compelling preclinical data supporting FLT3 inhibition as a therapeutic strategy in FLT3-mutated AML, until therapeutic benefit is proven from ongoing clinical trials in adults and children, up-front use of FLT3 inhibitors for childhood AML should be limited to clinical trials. However, for children with relapsed or refractory FLT3-ITD+ AML, limited therapeutic options (e.g. from anthracycline cardiotoxicity) and no access to a clinical trial, FLT3 inhibition could be considered to help achieve disease control, e.g., prior to HSCT or possibly even as a palliative strategy.


**Immunotherapy**

*Gemtuzumab ozogamicin*

Gemtuzumab ozogamicin (GO), a humanized anti-CD33 monoclonal antibody conjugated to the cytotoxic compound N-acetyl-γ-calicheamicin dimethylhydrazine, has been extensively investigated in adults and children over the past decade. Therapeutic targeting of CD33 has a sound rationale in AML, since approximately 80% of AML cases express CD33. High CD33 expression in childhood AML is associated with adverse disease characteristics such as FLT3-ITD and independently predicts poor outcome. Initial experience with GO monotherapy in children with relapsed/refractory AML demonstrated response rates of 53% (8/15 patients), with 6 patients proceeding to HSCT. Subsequent trials, including GO combined with chemotherapy and HSCT have demonstrated its safety. Monotherapy studies and case reports of GO in relapsed/refractory childhood AML have resulted in a number clinically meaningful responses, whilst phase II studies in combination with chemotherapy have shown similar results to historical controls. The recently reported multi-centre AML02 phase III trial included a randomization of GO (3 mg/m²) during induction II chemotherapy with cytarabine, daunorubicin and etoposide. Although the specific effects of GO versus no GO were not reported, GO in combination with ADE resulted in reductions in minimal residual disease (MRD) in 93% (27/29) of patients who had responded poorly to induction I, including all 8 patients with MRD > 25% after induction I. Of the 8 patients with MRD > 25%, half became MRD-negative with ADE+GO. Of the remaining 21 patients, who all had MRD > 1% after induction I, the ADE+GO combination reduced MRD in 19 (90%), with 9 patients becoming MRD-negative (43%). Paediatric phase III studies of GO in combination with conventional chemotherapy are ongoing.

The potential for veno-occlusive disease (VOD) of the liver following GO has been of
concern in adult patients, particularly in those patients proceeding to HSCT within 3.5 months of receiving 6 or 9 mg/m$^2$ GO. The recently reported UK NCRI AML15 trial (1,113 patients) administered a lower GO dose (3 mg/m$^2$) in adults and failed to show an increased risk of hepatic toxicity, even when administered within 120 days of HSCT. The successor trial, AML17 is randomizing adult patients to 3 vs. 6 mg/m$^2$. In pediatric studies liver toxicity has been less of an issue, and GO seems better tolerated in children.

Other dosing strategies of GO have also been examined, including fractionated therapy, facilitating cumulative doses of up to 27 mg/m$^2$ (ref 68-70). A phase I study in combination with busulfan and cyclophosphamide HSCT conditioning for 12 children with CD33$^+$ AML (median age 3 years, range 1-17) failed to identify any GO-associated DLTs at 3.0, 4.5, 6.0 or 7.5 mg/m$^2$ and had no 100-day transplant-related mortality.

In June 2010 the United States Food and Drug Administration (FDA) withdrew marketing approval for GO, based on lack of benefit in relapsed/refractory AML and increased induction mortality in adults. It has been highlighted, however, that the increased induction mortality associated with GO was relative to an unusually low mortality in the control arm of one of the studies the FDA based their decision on. Furthermore, subsequent data from 1,113 newly diagnosed patients treated on the UK NCRI AML15 trial have demonstrated a significant benefit of GO for patients with core binding factor (CBF) AML when given as a single dose of 3 g/m$^2$ during courses one and three. The biological explanation for this is somewhat unclear, since responses to GO were not related to blast CD33 expression.

Interestingly, children with CBF AML on the NOPHO-AML 2004 trial accounted for 35% (N = 17) of relapses, independent of whether or not GO (5 mg/m$^2$ x 2 doses) was given as post-consolidation therapy. Definitive results from larger paediatric trials of GO are
awaited. The recently reported French trial ALFA-0701 confirmed the survival advantage for adults with \textit{de novo} AML and favourable cytogenetics, but also for those with intermediate-risk cytogenetics. A notable feature of the ALFA-0701 study was the delivery of fractionated, higher cumulative doses of GO (3 g/m$^2$ days 1, 4 and 7 during induction and in two consolidation cycles). On the basis of these adult studies with fractionated dosing, further evaluation of GO in children is planned.

\textbf{Stem cell targeting}

\textit{Bortezomib}

The proteasome inhibitor, bortezomib, was initially approved for use in multiple myeloma. Pre-clinical studies of proteasomal inhibition in combination with idarubicin in AML demonstrated down-regulation of NF-κB and preferential cell death in leukaemic stem cells but not normal haemopoietic stem cells (HSCs). A single-agent phase I study in children demonstrated acceptable toxicity, with a RP2D of 1.3 mg/m$^2$ per dose when administered twice weekly for 2 weeks. Although NF-κB inhibition was observed in 2 of 5 evaluated patients, there were no objective clinical responses. Phase I studies of bortezomib (twice weekly for two weeks) in combination with chemotherapy in adult AML and paediatric ALL were also well tolerated, with predictable, chemotherapy-related toxicity. In the adult phase I combination study, nine patients were less than 60 years of age with relapsed AML, with the remaining 22 patients being over 60 years with untreated AML. Overall, 7/9 (78%) of relapsed patients and 15/22 (68%) untreated older patients achieved CR (including 3 CRp). In addition to administering Sorafenib to children with high-AR \textit{FLT3-ITD} AML, the current Children’s Oncology Group phase III trial (AAML1031, NCT01371981) is
randomising patients with *de novo* AML (including those with low-AR *FLT3*-ITD) to receive conventional chemotherapy with or without Bortezomib for all courses.

**Novel agents with promising data in adults**

**Aurora kinase inhibition**

Aurora kinases are a family of serine/threonine kinases with critical roles in mitosis.⁷⁶ Aurora A kinase plays a key role in centrosome maturation and mitotic spindle assembly whilst Aurora B kinase regulates the mitotic checkpoint, forms part of the chromosomal passenger complex (with inner centromere protein, Borealin and Survivin) and is required for final cytokinesis.⁷⁶ Although therapeutic success in solid tumours has been limited, Aurora kinase inhibitors have shown encouraging responses in leukaemia, particularly AML and Philadelphia-positive leukaemias.⁷⁷ A recent phase I/II trial of the selective Aurora B inhibitor, AZD1152 (barasertib) had an overall response rate of 25%.⁷⁸ The toxicity profile, including mucositis and myelosuppression, was reported as being manageable in an older population. FLT3 kinase is a secondary target of AZD1152⁷⁹ and although the *FLT3* status of patients on the trial was not reported, it is of interest that a number of responses occurred below the MTD and 55% of patients on the trial had cytogenetically normal AML (CN-AML). Given the high incidence of *FLT3* mutations in CN-AML, it is possible that the anti-FLT3 activity of AZD1152 may have contributed to efficacy in patients with *FLT3* mutations. A phase II/III study of AZD1152, alone and in combination with low-dose cytarabine in older adults (> 60 years), is currently ongoing (NCT00952588). A recent preclinical study has demonstrated that paediatric AML cell lines are sensitive to Aurora B knockdown by shRNA and small molecule inhibition with AZD1152.⁸⁰ One potential limitation of the AZD1152 compound is its delivery via a 7 day continuous IV infusion.
The selective Aurora A inhibitor MLN8237 (alisertib), has shown response rates of 17% in an adult phase II trial and preclinical activity in paediatric leukaemia. Of note, a recent preclinical study in acute megakaryoblastic leukaemia (AMKL) demonstrated that Aurora A inhibition with MLN8237 could induce polyploidization in both a Down Syndrome (DS) AMKL cell line (CMK) and primary blasts from children with non-DS-AMKL. MLN8237 also had in vivo activity against a non-DS-AMKL model. A paediatric phase II trial of MLN8237 in children with relapsed/refractory solid tumours and leukaemia is currently ongoing (NCT01154816, COG-ADVL0921). Similarly, the Aurora kinase inhibitor AT9283, which also has potent activity against ABL, JAK2 and FLT3 kinases, has shown encouraging responses in adult leukaemia trials and is currently being assessed in a paediatric phase I study (EudraCT No. 2009-016952-36; NCT01431664).

**MEK inhibition**

Mutations of N- and K-RAS in AML are examples of class I mutations, conferring a proliferative/survival stimulus. A recent comprehensive pre-clinical predictive biomarker analysis of 218 solid tumor and 81 haematological cancer cell lines revealed AML and CML cell lines as being particularly sensitive to the MEK inhibitor, GSK1120212. Amongst solid tumour cell lines, RAF/RAS mutations were predictive of sensitivity. In a panel of 12 AML cell lines, single-nanomolar IC$_{50}$’s were reported for all 6 RAS-mutant (N- or K-RAS) cell lines, as well as 2 RAS-wt cell lines. The paediatric cell line Kasumi-1 (RAS-wt) was resistant, although other paediatric cell lines, THP-1 (N-RAS mutant) and MV4-11 (FLT3-ITD$, RAS$-wt$), were sensitive. A phase I trial of GSK1120212 was reported at the 2010 ASH meeting, with 12 of 14 patients treated having AML (including 2 transformed from MDS). Preliminary anti-leukaemic activity was reported, with one patient achieving CR. A phase I/II study of GSK1120212 in AML is ongoing (NCT00920140).
Aminopeptidase Inhibition

Aminopeptidases play a key role in removing amino acids from cellular peptides, a process required for protein regulation and recycling. Aminopeptidase inhibition depletes intracellular amino acids required for new protein synthesis. AML cells have been shown to be sensitive to aminopeptidase inhibition, undergoing apoptosis, whilst normal bone marrow cells are less susceptible. The aminopeptidase inhibitor Tosedostat has shown promising activity in phase I/II trials of older adults with predominantly relapsed/refractory AML, with single-agent response rates of up to 27%. Tosedostat appears well tolerated with thrombocytopenia being the most common adverse event reported.

c-KIT Inhibition

Activating c-KIT mutations are common in paediatric and adult AML, particularly in CBF AML (incidence 21-54.5% with inv(16) and 17-46.8% with t(8;21)). Although c-KIT mutations confer an increased risk of relapse in adults with CBF AML, this does not appear to be the case in children. Murine models have demonstrated c-KIT mutations cooperate with AML1-ETO to cause aggressive AML in vivo, but are responsive to c-KIT inhibition with dasatinib. Dasatinib has also been shown to have single-agent activity against the t(8;21) paediatric cell line, Kasumi-1, which also harbours an N822K point mutation in the activation loop of c-KIT. Results of several ongoing clinical trials in AML, particularly those enriched with CBF patients, are eagerly awaited.

CXCR4 antagonism

The critical role of the bone marrow micro-environment, or “niche” in regulating normal and malignant haematopoiesis is becoming increasingly recognised. The CXCR4 antagonist,
plerixafor, has demonstrated efficacy in the mobilization of normal HSCs from the marrow for use in autologous transplantation in non-Hodgkin lymphoma and multiple myeloma.\textsuperscript{99,100} Plerixafor has also been demonstrated to be a feasible and effective second-line strategy in mobilizing HSCs in children.\textsuperscript{101,102} Based on pre-clinical data demonstrating AML blasts could be mobilized into the peripheral blood, a recent phase I/II study demonstrated the feasibility of combining plerixafor with cytotoxic chemotherapy in adults with relapsed/refractory AML. When used in combination with mitoxantrone, etoposide and cytarabine, plerixafor increased mobilization of AML blasts into the peripheral circulation.\textsuperscript{103} Overall CR rates of 46\% were achieved and no dose-limiting toxicities were observed.

**Epigenetic therapies**

Genes regulating DNA methylation and demethylation such as $\text{DNMT3A}$, $\text{IDH1/2}$ and $\text{TET2}$ are frequently mutated in adult AML and have prognostic significance.\textsuperscript{104-107} Consequently, targeting epigenetic processes has become an attractive therapeutic strategy in adult AML. The DNA methyltransferase inhibitors decitabine and azacitidine are currently being evaluated in clinical trials of adults with AML and paediatric evaluation programs are being designed. A recent analysis of 46 patients treated on two trials of decitabine has shown an improved response rate in patients with $\text{DNMT3A}$ mutations compared to those with wild-type $\text{DNMT3A}$ (CR rate 75\% (6/8) vs. 34\% (13/38) respectively, $P=0.05$).\textsuperscript{108} Studies of azacitidine alone and in sequential combination with lenalidomide have shown responses of approximately 50\% in older adults with newly-diagnosed AML compared to those with relapsed disease.\textsuperscript{109,110}

Another focus of epigenetic therapy has been histone deacetylase (HDAC) inhibition with compounds such as valproic acid, vorinostat and panabinostat. Widely used as an anti-
convulsant, valproic acid was first demonstrated to inhibit HDAC and cause partial
differentiation of the paediatric AML cell line Kasumi-1 over a decade ago.\textsuperscript{111} Preclinical
studies, including paediatric models, have also demonstrated single cytotoxic synergy with
cytarabine and clofarabine.\textsuperscript{112,113} Clinical responses to valproic acid alone or in combination
with cytarabine in adults with AML however, have been variable.\textsuperscript{114-116} It should be noted
that when combined with decitabine, valproic acid has been associated with
encephalopathy.\textsuperscript{117}

Although a single-agent randomised phase II trial of vorinostat in adults with relapsed or
high-risk untreated AML showed only minimal responses (1 CR from 37 patients)\textsuperscript{118} a recent
phase II trial of vorinostat in combination with idarubicin and cytarabine (IA) in patients with
AML and no CBF abnormalities resulted in an EFS of 47 weeks (range, 3 to 134 weeks) and
a CR rate of 76%. Although not randomised, these responses compared favourably to
historical data, the combination was reported as not excessively toxic compared to IA alone
and all 11 \textit{FLT3-ITD}\textsuperscript{+} patients responded (10 CR, 1 CRp).\textsuperscript{119} As discussed above, the relative
rarity of mutations in genes regulating DNA methylation in childhood AML may limit the
broader applicability of these agents for children, particularly if responses are confirmed to be
associated with mutational status.

Although adult clinical trials have only recently been initiated, it is worth noting one potential
epigenetic therapy that may be of significant importance to childhood leukemias with \textit{MLL}
gene rearrangements, given their relatively high incidence (AML 18%, ALL 8.3%).\textsuperscript{4} The
histone H3 methyltransferase, DOT1L, plays a key role in \textit{MLL}-fusion mediated
leukemogenesis (reviewed in Ref 120). A small molecule inhibitor of DOT1L, EPZ004777,
was recently shown to have \textit{in vivo} preclinical efficacy against \textit{MLL}-rearranged
leukemia. A phase I trial of an optimized compound, EPZ-5676, opened in September 2012 for adults patients with advanced hematological malignancies, including $MLL$-rearranged AML and ALL (NCT01684150).

**Finding and Hitting the Right Target in AML: Predictive and Pharmacodynamic Biomarkers**

Of critical importance for the rational and efficient development of targeted agents in cancer and leukaemia is the use of validated predictive and PD biomarkers. Predictive biomarkers are those that identify which patients are most likely to derive benefit from a given targeted agent, whilst PD biomarkers are needed to verify whether or not the desired molecular target is being modulated, and establish how target modulation relates to dose, toxicity and response. This information, together with careful examination of any resistance mechanisms or treatment failures, not only decreases the attrition rate of targeted therapeutics, but also improves the ongoing pre-clinical development of new agents.

FLT3 inhibitors provide arguably the best example of how robust predictive and PD biomarkers have been successfully applied in AML. It is well established that patients without activating mutations of $FLT3$ are less likely to benefit from FLT3 inhibition. \(^{33}\) Even with potent, second generation FLT3 inhibitors such as AC220, response rates in $FLT3$-WT patients are lower than in $FLT3$-ITD$^+$ patients (34 vs. 44\%). \(^{123}\) $FLT3$ mutational status therefore serves as a robust predictive biomarker for personalized AML therapy. Furthermore, results from trials of CEP-701 clearly demonstrate the importance of combining a predictive biomarker with a robust PD assay, since adequate FLT3 inhibition correlates with outcome for $FLT3$-mutated patients treated with CEP-701. \(^{45,47}\) An important PD assay developed for FLT3 inhibitors is the PIA assay. \(^{42}\) This assay involves incubation of a $FLT3$-
ITD\textsuperscript{+} cell line in the plasma of patients treated with FLT3 inhibitors. The degree of FLT3 inhibition seen \textit{ex vivo} for a patient at a given time-point during or after therapy is normalized to the amount of FLT3 phosphorylation observed when cells are incubated with that patient’s pre-treatment plasma. FLT3 ligand (FL) levels and protein binding can impact on the efficacy of FLT3 inhibition.\textsuperscript{46} The PIA assay is therefore superior to simply calculating the free drug concentration, since the plasma sample used to treat the cell line \textit{ex vivo} contains not only free drug, but also the patient’s actual FL and plasma proteins at that time-point. Using the PIA assay, it has recently been demonstrated that when adequate FLT3 inhibition is achieved in combination with chemotherapy, relapse rates are lower and overall survival can be improved.\textsuperscript{47}

The relatively small circulating blood volume and need for general anaesthetic when performing bone marrow aspiration can limit the number and nature of PD assays undertaken in paediatric leukaemia trials. Assays such as the PIA assay have been successfully adapted for use in children, such that smaller blood volumes are required, permitting use in phase I paediatric trials.\textsuperscript{124} Furthermore, the PIA assay has been successfully adapted and validated for therapeutic targets other than FLT3, with inhibition of phosphorylated histone H3 being used as a PD biomarker of Aurora kinase inhibition in a paediatric trial of AT9283 (EudraCT No. 2009-016952-36, NCT01431664).\textsuperscript{124}

**Future Directions and Trial Design**

Although the prognosis for children with AML has improved over recent decades, such improvements are largely attributable to more intensive use of cytotoxic chemotherapy and better supportive care.\textsuperscript{6} Although a number of novel therapeutic agents have been recently developed and show promising efficacy in adult AML, significant issues still exist for
executing early phase clinical trials of such drugs in children. Often, patients may progress rapidly between screening and commencing a novel therapy, by which time they may become ineligible with poor performance status or complicating conditions such as uncontrolled, invasive infection. Furthermore, patients relapsing soon after allogeneic HSCT may have poor organ function deeming them ineligible. One possible solution is to treat patients with primary refractory or first-relapsed AML with targeted novel agents in a “window” period prior to allogeneic HSCT. Several factors need to be considered for this approach though, including the likelihood of achieving CR with second or third line chemotherapy, the potential of the novel agent causing organ toxicity which could compromise the success of subsequent HSCT and the time required to screen, enroll and treat patients with the novel agent prior to HSCT.

Accelerating drug development for childhood AML will rely heavily on effective international collaboration, particularly if enrolment on targeted therapeutic trials is to be restricted to the relatively small number of patients with predictive biomarkers of response. Groups such as the Innovative Therapies for Children with Cancer (ITCC) European Consortium and the Therapeutic Advances in Childhood Leukaemia and Lymphoma (TACL) consortium have an existing focus on early-phase trials of novel agents for childhood leukaemia. Although legislative, regulatory and pharmaceutical supply barriers prevent the enrolment of children from outside Europe or North America on certain trials, attempts to facilitate truly international early-phase studies are ongoing. Novel approaches to early-phase trial design are also critical in accelerating drug development, particularly in children. Important strategies include phase I trials with expansion cohorts at the RP2D enrolling patients with predictive biomarkers, and adaptive randomised trials incorporating a number of new agents with the aim of “picking-the-winner” or “dropping-the-loser”. Such approaches
hold promise of reducing the number of large phase III trials needed and improving the likelihood of beneficial treatments being identified for malignancies like AML with increasingly heterogeneous biology. The important question of how much can be extrapolated from adult biology and trial data remains difficult to answer, hence defining the minimum dataset in children is an ongoing challenge.

Although significant challenges remain for novel drug development in paediatric oncology, particularly AML, the encouraging results of initial paediatric trials of therapies such as clofarabine and sorafenib, together with the large number of new agents in the pipeline is encouraging. Although survival rates in childhood AML are significantly better than adults with the disease, outcomes for children with AML are dramatically inferior compared with paediatric ALL. Improving outcomes in AML with cytotoxic chemotherapy alone is highly unlikely, given the current intensity of conventional therapy. Continued efforts to find effective novel therapies are therefore of critical importance if survival is to be improved and long-term morbidity reduced.
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Conflicts of Interest

ASM and ADJP are past and present employees respectively of The Institute of Cancer Research, which has a commercial interest in drug development programmes (see www.icr.ac.uk), and are subject to a “Rewards to Inventors Scheme” which may reward contributors to a programme that is subsequently licensed. ASM has received competitive research funding from Pfizer Inc., by way of a Pfizer Australia Cancer Research Grant.
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### Table I. Current clinical trials of new agents in childhood AML

<table>
<thead>
<tr>
<th>Class</th>
<th>Phase</th>
<th>Ages (years)</th>
<th>Target / Comments</th>
<th>Trial number</th>
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<tbody>
<tr>
<td>Nucleoside analogues</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clofarabine</td>
<td>I</td>
<td>0-28</td>
<td>In combination with thiotepa, topotecan and Vinorelbine</td>
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<td>In combination with cytarabine</td>
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<td>Clofarabine</td>
<td>I-II</td>
<td>0-30</td>
<td>In combination with cytarabine and TBI as conditioning for allo-HSCT</td>
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<tr>
<td>Clofarabine</td>
<td>I</td>
<td>0-18</td>
<td>In combination with cytarabine and liposomal daunorubicin</td>
<td>Dutch Trial Registry 1880</td>
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<td>FLT3 inhibitors</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Lestaurtinib (CEP-701)</td>
<td>III</td>
<td>0-60</td>
<td>FLT3-mutated patients randomised to CEP-701 or placebo after induction I; small numbers of paediatric patients.</td>
<td>ISRCTN55675535</td>
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<tr>
<td>Midostaurin (PKC412)</td>
<td>I-II</td>
<td>3 mo - 18</td>
<td>FLT3-mutated AML; MLL-rearranged ALL</td>
<td>NCT00866281 EudraCT No. 2008-006931-11</td>
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<td>Sorafenib</td>
<td>III</td>
<td>0-29</td>
<td>For FLT3-ITD+ patients with high-allelic ratio (&gt; 0.4)</td>
<td>NCT01371981 COG-AAML1031</td>
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<td>Quizartinib (AC220)</td>
<td>I</td>
<td>1mo - 21</td>
<td>Combination with cytarabine and etoposide</td>
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<td>Aurora kinase inhibitors</td>
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<td>AT9283</td>
<td>I</td>
<td>6 mo -18</td>
<td>IV, multi-kinase inhibitor (Aurora, ABL, FLT3, JAK2)</td>
<td>EudraCT No. 2009-016952-36 NCT01431664</td>
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<tr>
<td>MLN8237</td>
<td>II</td>
<td>1-21</td>
<td>Selective Aurora A inhibitor</td>
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<tr>
<td>Monoclonal antibodies</td>
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<tr>
<td>Gemtuzumab ozogamicin</td>
<td>III</td>
<td>0-29</td>
<td>Anti-CD33 mAb conjugated to cytotoxic calicheamicin</td>
<td>NCT01407757 COG-AAML0531*</td>
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<tr>
<td>Brentuximab vedotin</td>
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<td>≥ 6</td>
<td>Anti-CD30 mAb conjugated to anti-microtubule compound MMAE</td>
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<td>Proteasome inhibitors</td>
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<tr>
<td>Bortezomib</td>
<td>III</td>
<td>0-29</td>
<td>Bortezomib in combination with standard chemotherapy (ADE x 2, AE, A/Mitox)</td>
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<td>Other novel agents</td>
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<td>Panabinostat</td>
<td>I</td>
<td>8-21</td>
<td>HDAC inhibitor</td>
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<td>JAK1/2 inhibitor</td>
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<td>1-21</td>
<td>Akt inhibitor</td>
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<td>Obatoclax mesylate</td>
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<td>0-21</td>
<td>BCL-2 inhibitor, in combination with vincristine, doxorubicin and dexrazoxane</td>
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<td>Decitabine</td>
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<td>Hypomethylating agent as epigenetic priming for induction chemotherapy with ADE</td>
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<td>Fenretinide</td>
<td>I</td>
<td>0-21</td>
<td>Cytotoxic retinoid</td>
<td>NCT01187810</td>
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</table>
Abbreviations: TBI, total body irradiation; allo-HSCT, allogeneic haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; MLL, mixed lineage leukaemia gene; mo, months; mAb, monoclonal antibody; MMAE, monomethyl auristatin E; HDAC, histone deacetylase; ADE, cytarabine, daunorubicin, etoposide; AE, cytarabine etoposide; A/Mitox, cytarabine and mitoxantrone; *ongoing, but closed to recruitment.