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Emerging Pharmacotherapies for Adult Patients with Acute Lymphoblastic Leukemia

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Abstract: Acute lymphoblastic leukemia (ALL) treatment regimes are amongst the longest, most intensive and complex used in hemato-oncology. Despite this, while treatment of pediatric ALL is a success story, we are far from being able to ensure a durable response in adult ALL. This is not due to failure of induction therapy as a complete remission (CR) is achieved in over 90% of patients. However the challenge remains in ensuring a sustained remission. Furthermore in the face of relapsed disease, salvage therapies currently offer a poor chance of a good outcome. This article reviews the novel agents which show the most promise in the treatment of adult ALL.

Keywords: acute lymphoblastic leukemia, therapy, developments

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Introduction

In adult acute lymphoblastic leukemia (ALL) the 5 year overall survival (OS) has been estimated at 35%–47%^{1–7} and while there have been recent age-dependent improvements in survival,^{8,9} 5 year OS in adults compares poorly with that in childhood ALL where it has been estimated to be over 80%.¹⁰ This is not due to failure of induction therapy as a complete remission (CR) is achieved in over 90% of patients.¹ However the challenge remains in ensuring a sustained remission and in the design of effective salvage regimens.

The understanding of ALL disease biology has grown and we are gaining more insight into the genetic and pharmacogenetic factors associated with poor outcome. Additional therapeutic targets have become apparent and there is also better insight into how to use existing agents. This article outlines the current, emerging agents that show promise for improving the outcome of ALL treatment in adults.

Monoclonal Antibodies

Monoclonal antibodies have specificity for single epitopes and have found increasing uses in clinical medicine as both diagnostic tools as well as therapeutic agents.

Unmodified monoclonal antibodies

Rituximab

Rituximab has already had a considerable impact on the treatment of various B cell malignancies.¹¹ This chimeric anti CD20 IgG monoclonal antibody induces antibody-dependent and complement mediated cytotoxicity as well as apoptosis. Its efficacy is well established in B cell Non Hodgkin Lymphomas (NHL), particularly in combination with chemotherapy.¹²

Compared to mature B cells and their malignant counterparts, expression of CD20 is less commonly expressed on immature B cells and there is also a lower intensity of expression. While 80%–90% of Burkitt-type ALL cells express high levels of CD20, only 40%–50% of precursor B-lineage ALL cells express this antigen and with varying intensity.¹³ It is, however, important to note that no data are available to correlate a threshold for antigen expression and response to rituximab. Particularly intriguing is the observation that CD20 expression increases following induction chemotherapy in pediatric patients and it has been

postulated that this immunophenotypic alteration could be exploited with increased CD20 expression correlating to enhanced rituximab cytotoxicity *in vitro*.¹⁴

Hoelzer et al initially reported results of a chemoimmunotherapy regimen in Burkitts lymphoma (BL) or B acute lymphoblastic leukemia (B-ALL) in patients aged over 55. Twenty-six patients with B-ALL and a further 26 patients with mature B-ALL or BL received chemotherapy by the B-NHL2002 protocol with the addition of rituximab. For patients with precursor B-ALL, CR rate was 63% with a 1 year OS of 54% and in the mature B-ALL/BL group CR was 81% with a 1.5 year OS of 84%. Though follow up was short, this compared favorably with historical controls.¹⁸

The MD Anderson group studied 76 patients with BL and B-ALL evaluating the outcome of the addition of rituximab to Hyper CVAD (fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone plus methotrexate, high dose cytarabine). Rituximab was given at a dose of 375 mg/m² intravenously (IV) on Days 1 and 11 of hyper CVAD and on Days 2 and 8 of methotrexate and cytarabine. All but 4 patients had previously untreated ALL. Rituximab addition was not associated with increased therapy related toxicity. Overall, CR rates did not differ when rituximab was added but compared to historical controls, there was a significantly reduced relapse rate, an improved 3 year OS and complete remission duration (CRD), particularly in the over 60 age group.¹⁵ An update on the same patient group also revealed improved long term outcome with the addition of rituximab to therapy.¹⁹

An important point to bear in mind when evaluating these data is that neither of these two early studies were able to ensure that comparisons were made between patients with CD20 positive B-ALL (defined as greater than 20% expression) and CD20 negative B-ALL treated with rituximab or without. Since studies have shown that that CD20 expression is an independent poor prognostic factor,^{20,21} this important source of potential bias needs to be taken into account when interpreting the data.

In the German Multicenter Study Group for Adult ALL (GMALL) study 07/2003, younger patients with CD20 positive B-ALL were treated with rituximab according to risk group. In the standard risk group (definition of risk groups summarized by Hoelzer et al)²² rituximab improved the CR rate (from 57%



to 89%) as well as the 3 year OS (54% vs. 75%) and CRD (48% vs. 64%). Two thirds of patients in the high risk group proceeded to allogeneic stem cell transplant (SCT) and in this group rituximab was associated with an improved OS (40% vs. 75%).¹⁶

Another study from the MD Anderson included 282 adults and adolescents who were treated with standard or modified hyper CVAD, with the latter regimen incorporating anthracycline intensification, alteration to number of intrathecal treatments and extension of maintenance phase. If there was significant CD20 expression (again, defined as over 20% expression), rituximab was incorporated into the modified regimen.¹⁷ Median age was 41 years (range 13 to 83) and 21% of the study cohort was older than 60. CR was similar across the treatment groups, but in CD20 positive patients aged less than 60, the addition of rituximab to modified hyper CVAD resulted in an improved 3-year CRD (70% vs. 38%, $P < 0.001$) rate and OS (75% vs. 47%, $P = 0.003$) compared with standard hyper CVAD. In contrast, young patients with CD20 negative B-ALL did not have an improved outcome when treated with modified as opposed to standard hyper CVAD regimens (3-year CRD 72% with rituximab vs. 68% without, $P =$ not significant). BL and B-ALL patients aged over 60 did

not benefit from rituximab overall (3 year OS 28% with rituximab vs. 32% without $P =$ not significant), which may relate to a higher rate of death in CR.¹⁷

These data (summarized in Table 1) indicate that rituximab decreases risk of relapse and is associated with little excess toxicity. Of course, physicians do need to maintain vigilant to the rare, rituximab associated complications such as viral hepatitis reactivation and development of fatal progressive multifocal leucoencephalopathy related to JC polyomavirus. Two on-going phase 3 randomized controlled studies (UK National Cancer Research Institute UKALL14 and the French Group for Research in Adult Acute Lymphoblastic Leukemia GRAALL2005 trial) will confirm or refute the benefit of this agent in ALL.

Other anti CD20 antibodies are now available and may have different characteristics. Ofatumumab, for example has greater affinity for CD20, Veltuzumab is a humanized anti CD20.²³ These agents have been little studied in ALL to date.

Immunotoxin-Conjugated Antibodies

CD22 is a member of the sialic acid binding immunoglobulin-like lectin family of adhesion molecules and is expressed in virtually all malignant B cells. However, while the anti CD22 Epratuzumab has

Table 1. Studies comparing the efficacy of rituximab in adults with B-ALL.

Trial	Thomas et al ¹⁵		Hoelzer et al ¹⁶		Thomas et al ¹⁷			
Diagnosis	B-ALL/BL		B-ALL		B-ALL			
Evaluable patients	76		185		282			
Evaluable CD20 +ve patients	+		185		150			
Age	16–79		15–55		13–83			
Therapy	Hyper-CVAD	Hyper-CVAD + Ritux	GMALL 07/2003 (High risk)	GMALL 07/2003 + Ritux (High risk)	GMALL 07/2003 (standard risk)	GMALL 07/2003 + Ritux (standard risk)	Hyper-CVAD	Modified Hyper-CVAD + Ritux
Overall CR	85%	86%	40% [∞]	75% [∞]	93%	94%	94%	94%
Overall 3 yr CRD	66%	91%	*	*	48%	64%	40%	67%
Overall 3 year OS	53%	89%	32%	54%	54%	75%	45%	61%
Age >60 3year CRD	44%	100%	–	–	–	–	50%	45%
Age >60 3 yr OS	19%	89%	–	–	–	–	32%	28%
Age <60 3 yr CRD	73%	88%	–	–	–	–	38%	70%
Age <60 3 yr OS	70%	90%	–	–	–	–	47%	75%

Notes: [∞]Of the high risk patients who proceeded to SCT; *data not available; +not evaluated. Where data is available, OR and CR rates of CD20+ve patients are included in table only.

Abbreviations: ALL, acute lymphoblastic leukemia; BL, Burkitts lymphoma; CR, complete remission; CRD, CR duration; GMALL, German Multicenter Study Group for Adult ALL; Hyper-CVAD, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone plus methotrexate, high dose cytarabine; OS, overall survival.



shown limited clinical efficacy,²⁴ this molecule is an attractive target for conjugation with immunotoxins as bound molecules are rapidly internalized.²⁵

Combotox is a mixture of two immunotoxins prepared by coupling a ricin A chain to anti CD22 and CD19 antibodies. Seventeen patients aged 19–72 with refractory or relapsed ALL were given IV Combotox in a dose escalation regime. The maximum tolerated dose (MTD) was 7 mg/m² per dose or 21 mg/m² per cycle and vascular leak syndrome was the dose-limiting toxicity. Two patients developed reversible grade 3 elevations in liver function tests. The maximum plasma concentration (C_{max}) and half life ($t_{1/2}$) were both inversely proportional to blast count (C_{max} ranged from 66 to 1451 ng/mL and $t_{1/2}$ from less than 4 to 9.9 hours in patients with a high and low blast count respectively). Rapid reductions in blasts suggested specific cytotoxicity. One patient achieved partial remission and proceeded to allogeneic SCT.²⁶

Furthermore, data from a phase 1 trial in children suggested disease reduction prior to combotox may improve its efficacy.²⁷

The MD Anderson have reported early and promising results of Inotuzumab ozogamicin (IO), a CD22 monoclonal antibody attached to calicheamycin.²⁸ Forty patients aged 6 to 80 with relapsed or refractory ALL received 1.8 mg/m² IV over 1 hour every 3 weeks and overall at the time of reporting, 20 patients (56%) achieved a CR or complete marrow response. Of these 20, 12 were able to proceed to SCT. The most significant side effect was liver function abnormalities that were reported in 25% and severe in 11%. Two of these patients had liver biopsies that revealed periportal fibrosis.

This high CR rate in a heavily pretreated group of patients is noteworthy as is the high number of patients who proceeded to transplant. The MD Anderson has since observed that in the year prior to the availability of IO, 38% of ALL beyond second remission were transplanted while after IO became available, 67% were transplanted.²⁹ Between June 2010 and May 2011, 19 patients with a median age of 32 years (range 5–60) received an allogeneic SCT. With a median follow up of three months among surviving patients, a PFS of 59% at three months was observed.²⁹

Bispecific antibodies

Blinatumomab

CD19 is a pan B cell antigen and is therefore an attractive therapeutic target. Blinatumomab is a bispecific T cell engaging antibody composed of a single chain variable fragment (scFv) against CD19 coupled to an scFv against CD3 with the aim of activating T cells bound to CD19 expressing ALL blasts, thereby inducing perforin mediated death of the target cell. A phase 2 clinical study of blinatumomab in 21 adult patients with minimal residual disease (MRD) persistence or relapse has recently been reported.³⁰ Each cycle involved a continuous IV infusion of Blinatumomab at 15 µg/m²/24 hours for 4 weeks, followed by a two-week treatment free period. The most common side effect was lymphopenia (33.3%). A previous study in NHL used a higher dose of the drug and dose-limiting toxicity was neurological.³¹ At the lower dose used in this study, one patient experienced seizures and a further patient experienced syncope.

In terms of response, 16 subjects became MRD negative after one cycle of treatment; 12 of whom had been refractory to previous chemotherapy. This response was sustained in the majority of patients at a median follow up of 405 days. Eight patients progressed to allogeneic SCT. Of the 4 relapses, 2 were in isolated extramedullary sites and the other 2 patients relapsed with CD19 negative blasts. It was hypothesized that extramedullary relapses occurred in immunoprivileged sites where T cells are generally absent.³⁰

Interim results from a GMALL study evaluating blinatumomab in adult patients with relapsed or refractory B-ALL have also been reported.^{32,33} The first 18 patients reported were aged 18 to 77 and also received this agent as a 4-week continuous infusion, of varying doses in subsequent weeks of treatment, followed by a 2-week treatment free period with responders eligible to receive a further 3 cycles. At the time of reporting, 12 patients had reached a CR within 2 cycles of treatment including 3 with t(4;11) and one patient with a Philadelphia positive (Ph+ve) B-ALL. Four responders proceeded to SCT. Overall, 3 responders relapsed during treatment. One patient had a CD19 negative relapse post SCT. A further 2 patients relapsed during treatment with 1 patient having a CD19 positive extramedullary relapse and the other a



CD19 negative bone marrow relapse. While the most common side effect was fever and chills, 2 patients had severe side effects, which although reversible, necessitated discontinuation without completion of the first cycle. One of these patients had cytokine release syndrome (not seen in subsequent patients who were pretreated with dexamethasone or cyclophosphamide and a lower initial blinatumomab dose) and a second had encephalopathy and disorientation. Three further patients had reversible neurological events that required a temporary interruption of treatment.³³

As a result of these promising data a large registration study is now on-going in the European Union in patients with ALL who are MRD positive after 3 cycles of therapy.

Purine Nucleoside Analogues

Purine analogues form an important group of cytotoxic drugs which have proven efficacious in hematological malignancies.³⁴ Three novel purine nucleoside analogues have shown promise in the treatment ALL.

Nelarabine

Nelarabine is a soluble nucleoside analogue that is converted to 9-β-D-arabinofuranosylguanine (ara-G) after demethoxylation by adenosine deaminase. Ara-G, which is resistant to purine nucleoside phosphorylase (PNP) mediated phosphorylation, is intracellularly triphosphorylated to the active nucleotide ara-GTP which is then incorporated into DNA, resulting in chain termination, inhibition of ribonucleotide reductase and programmed cell death.⁴⁰ Ara-GTP appears to preferentially accumulate in malignant T cells and less so in B cells in which it also has a

shorter half-life, explaining its efficacy in T cell malignancies. Nelarabine achieved fast track approval by the FDA in October of 2005 for the treatment of patients with T acute lymphoblastic leukemia (T-ALL) and T lymphoblastic lymphoma (T-LBL) who have not responded to or have relapsed following treatment with at least two chemotherapeutic regimens. The use of nelarabine for T-ALL in adult patients has been studied in a number of trials, which are summarized in Table 2.

Kurtzberg et al studied dose escalations of nelarabine in multiple hematological malignancies in both adult and pediatric patients establishing a MTD of 40 mg/kg in adults. Of 13 evaluable adults with T-ALL/T-LBL, 15% achieved CR and 62% achieved PR. However, the drug was associated with significant neurotoxicity, which was mostly reversible including weakness, ataxia, and confusion. Coma was observed in 3 of 4 adults.³⁵

A more focused CALGB 19801 study examined the outcome of using nelarabine only in relapsed or primary refractory T cell malignancies, primarily in adults. The dose used in this study was 1.5 g/m² on days 1, 3 and 5, repeated in a 21-day cycle. Patients with residual disease after the first course were offered a second and patients who achieved a CR after the first cycle were eligible to receive another two courses. Thirty-nine patients were evaluable and 31% of T-ALL and a further 31% of T-LBL patients achieved a CR. This dosing schedule was also better tolerated with a markedly lower incidence of grade 3 or 4 neurotoxicity compared with that reported in Kurtzberg et al.³⁶

A National Cancer Institute phase 2 study of nelarabine in Non-Hodgkins Lymphoma included

Table 2. Trials of Nelarabine monotherapy in relapsed or refractory adult T-ALL/LBL.

Trial	Kurtzberg et al ³⁵	DeAngelo et al ³⁶	Goy et al ³⁷	Czuczman et al ³⁸	Gokbuget ³⁹
Total evaluable patients	93	39	17	19	126
Evaluable adults with T-ALL/T-LBL	13	39	8	19	126
Diagnosis	T-ALL/T-LBL	T-ALL/T-LBL	T-LBL	Cutaneous and peripheral T-LBL	T-ALL/T-LBL
Age range	22–75	16–66	33–81	33–69	18–81
ALL/LBL CR	15.0%	30.8%	25.0%	0%	36%
ALL/LBL PR	62%	10.3%	25.0%	10.5%	10%

Abbreviations: ALL, acute lymphoblastic leukemia; CR, complete response; LBL, lymphoblastic lymphoma; PR, partial response.



13 patients with T-LBL. Nelarabine was administered at a dose of 1.5 g/m²/day on days 1, 3 and 5 in a 28-day treatment cycle and up to 6 cycles were administered. Only 17 of the 23 patients were evaluable and overall 12% and 38% of patients achieved a CR and partial response (PR) respectively. Four of 8 evaluable patients with T-LBL responded (2 to CR and 2 to PR).³⁷ The CALGB 59901 trial further evaluated nelarabine in cutaneous and peripheral T-LBL. Of 19 evaluable patients only 2 achieved a PR and none a CR. These poor results may reflect varying histological subtypes of the disease or varying disease biology compared to the other studies.³⁸

The largest trial so far of nelarabine monotherapy in the setting of relapsed or refractory T-ALL or T-LBL in adults is the recently published GMALL exploratory phase 2 study.³⁹ The aim was to evaluate efficacy and tolerability of nelarabine in adult patients and the feasibility of subsequent SCT. One hundred and thirty-three patients aged 18–81 were recruited and administered nelarabine using the CALGB dosing regime. Study treatment was stopped in those who had not achieved a CR after two cycles and patients in CR, eligible for a SCT, and with an available donor were removed from the protocol. Overall, after 2 cycles, 36% and 10% of patients achieved a CR and PR respectively. A small number of patients had a third cycle and no additional CRs were obtained from this extra treatment. Interestingly, 13 patients entered the study a second time in relapse (including 8 after SCT) and 5 of these achieved a CR after 1–2 cycles. Myeloid blasts were associated with 5 patients that didn't respond in this group. Of particular relevance in interpreting the results of other trials, none of the patients with the initial diagnosis of T-LBL achieved a CR.

Despite the heavy pretreatment of this cohort, toxicity was low with overall 16% neurotoxicity and 7% grade 3–4 toxicity. There was also an acceptable level of grade 3–4 neutropenia (37%) and thrombocytopenia (17%).

In this GMALL study, 80% of the 45 patients who achieved a CR from nelarabine monotherapy proceeded to SCT. Three year OS in this transplanted group was 36% compared to 0% in those achieving CR with nelarabine but not receiving SCT.³⁹

Further work is needed to determine the optimal use of nelarabine in order to maximize its

antileukemic affect while minimizing toxicity. This is likely to involve incorporation of nelarabine into combination regimens and broadening its indication beyond relapse. There is a recently published study of 7 children with relapsed or refractory T cell leukemia or lymphoma who were treated with nelarabine, etoposide and cyclophosphamide. All subjects achieved a response including a CR in all 4 patients with T-ALL and the one patient with bilineage ALL/acute myeloid leukemia (AML).⁴¹

The ongoing UKALL14 and forthcoming GMALL 08/2011 studies will both look at the role of nelarabine at induction, in UKALL14 administration will be randomized.

Clofarabine

Clofarabine is another nucleoside purine analogue with similarities to other drugs of this class as well as some unique qualities. It is phosphorylated in the intracellular compartment to its active triphosphate form and combines the fludarabine-like ability of inhibiting DNA polymerase by terminal incorporation into DNA and the cladribine-like quality of inhibiting ribonucleotide reductase.⁴⁷ Clofarabine is also resistant to PNP and adenosine deaminase and appears to directly affect the mitochondrial membrane leading to release of apoptosis inducing factors.⁴⁸

A significant body of evidence supports its use in chronic lymphocytic leukemia (CLL) and AML and it is also licensed for use in relapsed and refractory pediatric ALL who have had two previous lines of therapy.^{49–51} However, the evidence for clofarabine, summarized in Table 3, in adult ALL is more limited.

Kantarjian and colleagues explored Clofarabine monotherapy in a phase 1 followed by a phase 2 trial and although the number of ALL patients were small, there was a limited response.^{42,43} Clofarabine was administered as an hour-long intravenous infusion daily for 5 consecutive days and the MTD in acute leukemia was 40 mg/m² per infusion. The most common grade 3–4 side effect was hepatotoxicity. Eighty-one percent of patients developed febrile neutropenia and 50% had documented infection during treatment. There were no deaths directly related to drug toxicity. Two of the 12 patients with ALL had a CR.

Studies have examined combinations of clofarabine in conjunction with cyclophosphamide and cytarabine in adult ALL. Cyclophosphamide is an alkylating

**Table 3.** Trials of clofarabine in relapsed or refractory adult ALL.

Trial	Kantarjian ⁴² (monotherapy phase 1)	Kantarjian ⁴³ (monotherapy phase 2)	Faderl et al ⁴⁴ (+cytarabine)	Karp et al ⁴⁵ (+cyclophosphamide)	Advani et al ⁴⁶ (+cytarabine)
Total evaluable adults	51	62	32	18	36
Adults with ALL	13	12	2	6	36
Diagnosis	ALL⁺	ALL⁺	ALL⁺	T-ALL (n = 1), Ph+ve ALL (n = 1), B-ALL (n = 6)	B-ALL (80.6%)/ T-ALL (19.4%)
Age range	19–78	19–82	18–84	27–67	20–68
ALL CR	7.7%	16.6%	0%	50.0%	16.7%
ALL PR	7.7%	0%	0%	16.7%	0%

Note: ⁺No further diagnostic detail provided.

Abbreviations: ALL, acute lymphoblastic leukemia; CR, complete response; Ph+ve, Philadelphia positive; PR, partial response.

agent that mediates interstrand crosslinking of DNA and CLL cells have the capability of repairing this in vitro. Pretreatment of CLL cells with clofarabine interferes with this capability therefore increasing apoptosis.⁵² Following this preclinical data, the treatment schedule designed for a phase 1 clinical trial concerning this particular chemotherapy combination was clofarabine (at 10 or 20 mg/m²) on days 1, 3, 8, 10 administered two hours prior to cyclophosphamide (400 mg/m²). Of the 18 patients in this study, age ranged from 21 to 67 years with a median age of 51 and 6 had ALL. Four of these 6 patients had adverse cytogenetics, and all patients in the study had refractory leukemia with multiple prior therapies. This chemotherapy combination did result in increased DNA damage and apoptosis but was, however, significantly myelosuppressive with a median time to marrow recovery of 45 days and one third of patients on the higher dose of clofarabine aplastic for over 60 days. Four patients died during therapy with 1 patient who had irreversible aplasia without recurrent leukemia at day 100 and multiorgan failure. Overall an impressive 50% of ALL patients achieved CR and 16.7% a PR, but none of these patients proceeded to SCT.⁴⁵

In vitro data also indicated that clofarabine would increase intracellular cytarabine concentrations thereby augmenting its cytotoxicity.⁵³ However, in contrast to the clofarabine and cyclophosphamide combination, clofarabine and cytarabine therapy did not result in a notable clinical benefit in the Southwest Oncology Group Study S0530 phase 2 trial. Thirty-six patients with relapsed or refractory disease were

included, induction therapy consisting of clofarabine 40 mg/m²/day and cytarabine 1 g/m²/day on days 1–5. The most common Grade 3 or greater non-hematologic toxicities were infection (64%) and metabolic or laboratory abnormalities (33%). Ten deaths occurred during treatment, 7 of which were attributable to therapy. Only 17% achieved a CR, half of which also had incomplete count recovery.⁴⁶

Future work will define optimal combination therapies and dosing to maximize the antileukemic affect of clofarabine while minimizing its toxicity.

Forodesine

Forodesine, a PNP binding drug, has a unique mechanism of action which does not depend on incorporation into DNA to exert its cytotoxic effects.⁵⁴ Preclinical data indicate that forodesine is selectively cytotoxic to T-ALL cells.⁵⁵

PNP is an enzyme that degrades deoxyguanosine (dGuo), which is continuously produced by the body as a by-product of DNA breakdown during cellular turnover. Inhibition of PNP results in accumulation of dGuo that is in turn phosphorylated to deoxyguanosine triphosphate (dGTP). Intracellular accumulation of dGTP then results in cell cycle arrest and apoptosis via an ill-understood mechanism.^{56,57}

A phase 1 study included 5 patients of whom 2 patients had T-ALL in first relapse. Forodesine was administered intravenously over 30 minutes at a dose of 40 mg/m² for five days (once on day 1 and then twice daily for the remaining 4 days). C_{max} was achieved at the end of infusion, median t_{1/2} was 11 hours and the medication was mainly renally cleared. The most



common side effect was grade 3–4 neutropenia. Both patients had a transient improvement in blast count but there was no objective response in either.⁵⁸

Further study is needed to determine the potential beneficial therapeutic effect of forodesine in ALL.

NOTCH 1 Inhibitors

NOTCH receptors play a key role in mediating multiple stages of T cell development. This molecule consists of an extramembrane portion that attaches to activating ligands, resulting in proteolytic cleavage of the receptor complex that then releases an intracellular domain to translocate into the nucleus and induce expression of NOTCH 1 target genes.⁵⁹

The first link between NOTCH1 and T-ALL was the demonstration that the t(7;9)(q34;q34.3) translocation resulted in a truncated NOTCH1 receptor. This receptor was either more vulnerable to proteolytic cleavage and thus activation, or lacked a transmembrane portion to anchor the intracellular domain resulting in constitutive gene activation.^{60,61} It was soon discovered NOTCH1 mutations were not isolated to this particular translocation but that over 50% of human T-ALL samples have one of a variety of mutations to the regulatory portion, causing ligand independent receptor activation or ligand hypersensitivity.⁶² This discovery established NOTCH1 as a potential therapeutic target.

One of the two key activating proteolytic steps which cleaves the NOTCH1 molecule on ligand binding to release the intracellular domain involves the enzyme γ -secretase. This same enzyme is also involved in the pathogenic deposition of amyloid fibrils in the brain found in patients with Alzheimer's disease. Hence, γ -secretase inhibitors (GSI), originally designed for Alzheimer's therapy have been studied in T-ALL.

Preclinical models were promising with inhibition of the NOTCH1 receptor by GSIs resulting in decreased growth and proliferation characterized by G₀/G₁ cell cycle arrest.^{61,62} However a phase 1 trial of the GSI MK-0752 in patients with T-ALL was less encouraging. Six adult and 2 pediatric patients with leukemia (7 with T-ALL and 1 with AML) received MK-0752 orally once a day at 150, 225, and 300 mg/m². Only 1 patient achieved a transient clinical response but with significant gastrointestinal toxicity.⁶³ Intestinal endothelium seems to be particularly

sensitive to NOTCH inhibition with an accumulation of mucus secreting goblet cells with GSIs. In addition, where GSIs appear to induce a significant response with marked apoptosis in murine ALL cell lines, this is not reflected in human ALL cell lines where only a cytostatic effect is seen.^{61,62,64} Furthermore, as NOTCH1 receptor stimulation promotes cell growth via numerous mechanisms, additional mutations in any of these downstream pathways would conceivably ameliorate NOTCH1 inhibition and it is therefore not surprising that resistance to GSIs is prevalent.⁶²

Few of our current standard cytotoxic therapies are used in isolation and there is early evidence that targeting both NOTCH1 activation as well as critical downstream steps can have a powerful antileukemic effect. Concurrent inhibition of AKT,⁶⁵ Hedgehog and Wnt,⁶⁶ cyclin D kinase,⁶⁷ PI3K-AKT-mTOR pathway^{65,68} need more investigation. In addition, study of a glucocorticoid resistant ALL cell line showed that, in combination, GSIs and glucocorticoid induces apoptotic cell death and has the added positive effect of protecting mice from the gastrointestinal toxicity typical of GSIs.⁶⁹

mTOR Inhibitors

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that, through its interactions with a number of signaling pathways, functions as a key regulator of cell growth, protein synthesis and cell cycle pathways. Many hematological malignancies have aberrant expression of mTOR and *in vitro* as well as *in vivo* murine studies have shown that mTOR inhibitors (MTIs) (sirolimus, temsirolimus, everolimus) have activity against both B- and T-ALL cells.⁷⁰ MTIs are widely used for immunosuppression and are relatively well tolerated.

Two phase 1/2 trials have investigated MTIs in the setting of relapsed hematological malignancies in adults which included two patients with ALL who tolerated therapy but without any objective response.^{71,72}

Resistance to MTIs may occur by up-regulation of other intermediary signals in the PI3K/AKT/mTOR signaling pathways. Combinations of inhibitors or combination of MTIs with chemotherapy or steroids have been explored in pre-clinical work and need further study to determine their therapeutic value.^{73–79}



Sorafenib

Sorafenib, a multi-targeted tyrosine kinase inhibitor with activity against RAF kinase, VEGF receptors, both wild type and internal tandem repeat mutated FLT3, PDGF receptors, c-KIT, and RET kinase⁸⁰ is licensed for the treatment of renal cell and hepatocellular carcinoma and is being evaluated in numerous malignancies.^{81–84}

Preclinical work in B- and T-ALL cells suggest that sorafenib induces cell cycle arrest by directly inhibiting Erk, mTOR and Akt, and induces apoptosis by cleavage of caspases 3, 7 and PARP.⁸⁵ Two patients with ALL were treated with sorafenib in a dose escalation manner in a phase 1 trial of relapsed or refractory leukemia.⁸⁶ In this study the maximum tolerated dose was 400 mg BD orally for 21 days. At this dose 48% of patients experienced grade 3–4 toxicity overall (most common 20% experienced fatigue and 14% febrile neutropenia). One of the 2 ALL patients cleared their peripheral blasts and achieved a near 50% reduction in marrow blasts after 2 cycles. The authors note that this patient had a mixed lineage leukemia (MLL) rearrangement with translocation (4;11), which has been associated with over expression of wild type FLT3 and in vitro sensitivity to FLT3 inhibitors.⁸⁶

Aurora kinase Inhibitors

Three subtypes of Aurora kinases (A, B and C) make up a family of highly conserved serine-threonine protein kinases that have a key role in several stages of mitosis. Mutations in Aurora kinases resulting in their over expression or amplification have been observed in a wide range of malignancies.⁸⁷ Aurora kinase inhibitors (AKI) attach to the ATP binding site, vary in their specificity for these target enzymes and most AKIs also have the capability of multi-kinase inhibition against ABL, JAK2 and FLT3. Their ability to inhibit ABL have made AKIs attractive agents for Ph+ve leukemias and it has also been observed that many AKIs can overcome resistance to tyrosine kinase inhibitors (TKI), even when arising from the T315I mutation.⁸⁸

One of the first AKIs to be investigated in Ph+ve leukemia was MK-0457. Initially 3 adult patients with T315I mutated chronic myeloid leukemia (CML, 2 patients) and ALL (1 patient, relapsed ALL, aged 63) were administered MK-0457 in a

continuous infusion for 5 days at 2–3 week intervals. Significant BCR-ABL inhibition occurred at doses of 20 mg/m²/hour. The only reported side effect was reversible pancytopenia.⁸⁹ This was followed by a phase 2 trial which was closed early following the discovery of QTc prolongation in one subject.⁹⁰

A second AKI, AT9283, has pan-Aurora, ABL, FLT3 and JAK2 kinase inhibitor activity. In a phase 1 trial which included patients with ALL, there was response reported in patients with AML and CML only.⁹¹ XL228 is a multi kinase inhibitor and is currently the subject of a phase 1 trial of 27 patients with CML or Ph+ve ALL who have been either resistant or intolerant of two TKIs or have the T315I mutation. XL228 was administered in 1 hourly infusions once or twice a week and the main side effect observed was increased insulin and glucose levels. An initial report has described clinical activity in 17 of a total 27 patients, evidenced by improvement in white cell or platelet count or greater than 1 log reduction in BCR-ABL levels, at doses of 3.6 mg/kg and higher. Seven of the 17 responders have the T315I mutation.⁹² A fourth agent, Danusertib has pan-Aurora and ABL inhibitory activity and is in a phase 1 trial of 23 patients with relapsed CML or ALL. There are 11 patients with ALL included in the study and patients are administered 3 hourly IV infusions for 7 days every 2 weeks in a dose-escalating regime. An early report from this study has described response in 6 patients.⁹³

There is also a growing body of preclinical evidence that AKIs also have increased cytotoxicity when used in combination with TKIs, conventional chemotherapeutic drugs or other novel agents such as histone deacetylase inhibitors.^{94–97}

The multi kinase inhibitory ability of AKIs has the theoretical advantage of greater cytotoxicity and also decreased risk of leukemic cells evolving resistance. However, we are yet to elucidate the key biological targets in Ph+ve ALL which mediate clinical response.⁹⁸ Until we do understand this, we are unlikely to design optimal treatment regimes and drug combinations that maximize the antileukemic effect while minimizing the toxicity of AKIs.

Histone Deacetylase Inhibitors and Hypomethylating Agents

Malignant phenotype is not determined by genotype alone. ‘Epigenetic’ modifications influence



gene function without altering the underlying DNA sequence.⁹⁹ As an example, aberrant methylation of cytosine residues, particularly in and around so-called CpG islands can result in silencing of particular gene sequences including tumor suppressor genes and promote tumor formation.¹⁰⁰ Epigenetic modifications are common in ALL, and increased gene methylation has been associated with relapse and poorer prognosis.^{101,102} Such modifications may also play a role in ALL pathogenesis. For example, MLL mutated ALL can result in a translocation to produce the MLL-AF4 protein that recruits the histone methyltransferase DOT1L. This enzyme methylates the histone H3 lysine 79 (H3K79) and accordingly there is reduced expression of several critical genes that have this altered histone.¹⁰³ A second epigenetic modification seen in ALL is hypermethylation. In infants, it has been demonstrated that one of the domains required to produce an MLL oncoprotein with leukemic potential is a sequence with homology to the regulatory portion of eukaryotic DNA methyltransferase (DNMT1) (termed the MT domain). MLL MT recognizes the unmethylated CpG nucleotide sequences thereby silencing gene expression.¹⁰⁴

Histone deacetylase inhibitors (HDACi) are able to modify chromatin structure and enhance DNA transcription. While a significant body of preclinical data have shown HDACis to be cytotoxic to ALL cells,¹⁰⁵ a number of phase 1 trials of HDACis in adult leukemic patients have included only small numbers of patients with ALL and it has not yet been determined if this class of drug will be useful in the treatment of this disease. A phase 1 study of LBH589 included 1 patient with ALL¹⁰⁶ and a phase 1 study of vorinostat included 2 patients with ALL.¹⁰⁷

It has also been hypothesized that the ability of HDACis to open the chromatin configuration could allow better DNA access to cytotoxics as well as up-regulating DNA topoisomerase interaction thereby sensitizing leukemia cells to anthracyclines.¹⁰⁸ Hence, most of the ongoing clinical trials of HDACis in ALL consist of this class of drug in a combination regime. Mummery et al have extensively reviewed the epigenetic abnormalities and the currently studied HDACis in relation to ALL.¹⁰⁵

There has also been interest in hypomethylating agents. In vitro, decitabine has significant activity against ALL derived cell lines.¹⁰⁹ A phase 1 study has

been reported involving 39 patients (aged 4–67) with relapsed disease who were treated with an escalating dose of decitabine alone followed by decitabine combined with hyper CVAD in those who either did not respond or who lost their response to the single agent.¹¹⁰ Twenty-three percent of patients achieved a transient CR with decitabine alone and the optimal dose was determined to be 60 mg/m² IV daily for 5 days every fortnight. Half of patients who were treated initially with decitabine alone were then treated with hyper-CVAD as well. Fifty-two percent of patients achieved a response with this combination for a median duration of 4 months. The optimal dose when used in combination was 40 mg/m² IV given for 5 consecutive days with each hyper CVAD cycle. The authors reported no significant toxicity with decitabine used alone or in combination. While these results may show some promise, the responses do seem short lived. We await further data of this class of agents in the treatment of ALL, with particular interest in whether decitabine facilitates patients proceeding to SCT and if other combination regimes can impact long term survival.

Mitoxantrone

Mitoxantrone is a type II topoisomerase inhibitor, has a favorable chemosensitivity profile in relapsed ALL and has a reported B cell specific affect.^{111,112} In the ALL R3 trial, 239 pediatric patients in first relapse aged 1–18 were randomized to have either mitoxantrone or idarubicin at induction. The randomization was terminated early by the Data and Safety Monitoring Committee because there was a clear improvement in relapse rate in the mitoxantrone arm. Three year OS was 45.2% in the idarubicin group and 69% in the mitoxantrone group with a similar improvement to 3-year progression free survival (36% to 65%). This improvement was achieved even though the overall toxic affects were lower in the mitoxantrone group, though there was a noted increased incidence of hematological toxicity in the later phases of treatment.¹¹³

So far, mainly clinical studies in adult ALL patients have been detailed in this article. However in the ALL R3 trial, mitoxantrone translated into a survival advantage of over 20% in this pediatric cohort, which is one of the most significant improvements to outcome following a single modification of treatment.



Table 4. Summary of emerging therapies in adult ALL.

Agent	Mechanism	Evidence in adult ALL	Significant toxicity	Current place in ALL therapy	Future directions
Rituximab	Anti CD20 monoclonal antibody	Phase 2: Reduces relapse in CD20+ve ALL with a 3 yr CRD of 60% in under 60 year olds when used in combination with modified hyper CVAD ¹⁷ Phase 2 (Early data): Promising as a single agent in relapsed, refractory B-ALL ²⁸ , with CR in 56% and >60% proceeding to SCT ²⁹	Liver function abnormalities in 25%, severe in 11% ²⁸	Not licensed	<ul style="list-style-type: none"> • Further assessment in 1st induction in CD20+ve ALL • Assess efficacy of 2nd generation monoclonal antibodies • Further assess efficacy in adult ALL • Further assessment of other immunotoxins eg, Combotox particularly in combination with cytotoxics
Inotuzumab ozogamicin	Immunotoxin: CD22 monoclonal antibody attached to calicheamycin			Not licensed	<ul style="list-style-type: none"> • Further assess in MRD persistence or relapse • Consider use in 1st induction
Blinatumomab	Bispecific T cell engager	Phase 2: In MRD persistence or relapse, 76% became MRD negative ³⁰ and early data suggesting efficacy in relapsed or refractory B-ALL (67% CR) ³² Phase 2: 36% achieved CR in relapsed T-ALL ³⁹	Neurotoxicity: Seizures in 2/21 patients ³⁰	Not licensed	
Nelarabine	Nucleoside Purine analogue		Neurotoxicity. 16%, 7% grade 3–4 ³⁹	FDA approved for T-ALL/T-LBL failed 2 previous lines of therapy	<ul style="list-style-type: none"> • Earlier use in adult T-ALL (1st relapse or 1st induction) • Combination regimens • Assess use as maintenance in patients ineligible for SCT • Further investigate other nucleoside purine analogues • Needs further assessment
Sorafenib	Multi targeted tyrosine kinase inhibitor	Phase 1: Limited so far ⁸⁶		Not licensed	
Sirolimus	mTOR inhibitor	Phase 1: Limited response to monotherapy ⁷²		Not licensed	<ul style="list-style-type: none"> • Assess combination regimens
Gamma secretase inhibitors	NOTCH inhibitor	MK-0752 Phase 1: Poor clinical response ⁶³	Gastro-intestinal toxicity	Not licensed	<ul style="list-style-type: none"> • Investigate combination regimens
Aurora kinase inhibitors		Phase 1: Limited clinical response so far ^{89,91–93}	MK-0457: Q.Tc prolongation ⁹⁰	Not licensed	<ul style="list-style-type: none"> • Further investigate use in Ph+ve leukemias • Combination regimens • Front line therapy with TKIs • Define efficacy as monotherapy • Optimal combination regimens
Histone deacetylase inhibitors		Limited clinical response so far but phase 1/2 trials ongoing ^{106,107}		Not licensed	

(Continued)



Table 4. (Continued)

Agent	Mechanism	Evidence in adult ALL	Significant toxicity	Current place in ALL therapy	Future directions
Decitabine	DNA hypomethylating agent	Phase 1: Efficacy as single agent (23% CR) or in combination with hyper CVAD (52% CR) at inducing short lived responses ¹⁰		Not licensed	<ul style="list-style-type: none"> • Assess long term outcome as single agent or in combination. • Investigate outcome of subsequent SCT • Define efficacy in adult ALL
Mitoxantrone	Topoisomerase inhibitor	ALLR3 study: In pediatric patients, reduced relapse rate and increased OS at induction compared to idarubicin (3 yr OS 45% vs. 69%) ¹¹³		Not licensed	

Abbreviations: ALL, acute lymphoblastic leukemia; CR, complete response; CRD, CR duration; LBL, lymphoblastic lymphoma; MRD, minimal residual disease; OS, overall survival; Ph+ve, Philadelphia chromosome positive; SCT, stem cell transplant.

Similar work in adult ALL is required to determine if mitoxantrone is also beneficial in an older age group.

Conclusion

There have been significant clinical responses to a number of novel agents (summarized in Table 4). Notably, nelarabine in T-ALL, as well as rituximab and blinatumomab in B-ALL are promising and are undergoing large international phase 2 and 3 studies in earlier phases of the disease. By contrast, considerably more clinical study is required to determine what role these as well as immunotoxins, AKIs, HDACis, hypomethylating agents, GSIs, MTIs, mitoxantrone and other purine nucleoside analogues have in the treatment of adult ALL. It is important to be mindful that although our attention is often optimistically directed towards new drugs, improved responses have been recently achieved with conventional and easily accessible agents whose use is established in other malignancies (eg, mitoxantrone and rituximab).

Furthermore, the majority of agents will unlikely realize their optimal clinical potential as monotherapy and an increasing knowledge of disease biology as well as an understanding of the mechanisms by which these agents exert their antileukemic affect will enable treatment regimes to be rationalized. Given the complexity of this task, this can only be achieved with international collaboration.

In contrast to the previously practiced ‘one size fits all’ approach, current treatment principles are progressively more individualized with early risk stratification and targeted therapy. As accurate assessment of individual risk becomes increasingly possible, the therapeutic landscape may change considerably. It will therefore be important that our study designs recognize this and incorporate novel end points such as MRD quantification as well as high quality correlative science projects.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed



patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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