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Acute myeloid leukaemia - therapy - past, present and future

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Abstract

Acute myeloid leukemia (AML) is characterized by genetic aberrations and a variable response to therapy which has made treatment of AML challenging. The objective of this paper is to review conventional treatments and their development, phase I-III clinical trials of new agents, novel pathways where future interventions may have therapeutic potential, and clinical trial assessment in AML. This study showed that a detailed understanding of the molecular changes associated with chromosomal and genetic abnormalities is necessary to pilot new therapy design. Although several deregulated proteins and genes have been identified, their diversity among AML patients have made it difficult to identify a single substance that can hit these diverse targets . New agents have shown promise but there remains a huge need to be met for effective and targeted therapies to be successful.

Introduction

Chemotherapy, irradiation and haematopoietic stem cell transplantation (HSCT) are now standard therapies in acute myeloid leukaemia (AML) (1). Chemotherapies, anti-metabolites blocking DNA/RNA synthesis, have been a corner stone for three decades. However they have numerous side effects and limitations in efficacy (2). HSCT is similarly challenged by toxicity and efficacy(3). The overall 5-year survival from AML remains poor, particularly in adults and in the elderly (4). Genomic and proteomic technologies have provided opportunities for development of targeted therapies through improved understanding of molecular biology, and better characterization of AML subgroups (5-7). New agents are in phase I-III clinical trials. Several have been rejected as ineffective or unsafe but a small number have demonstrated potential (8). However, none stand alone as mono-therapies or more effective than standard chemotherapy in low- and intermediate-risk patient groups, and continue to be assessed for adjuvant properties. This paper will review conventional treatments and their development, phase I-III clinical trials of new agents, novel pathways where future interventions may have therapeutic potential, and clinical trial assessment in AML.

Drug therapies

Chemotherapy

Chemotherapy protocols are divided in to two stages. The first, 'Induction', aims to reduce diseased cells to undetectable levels (complete remission (CR)). The second, 'Consolidation' (or post-remission), is the elimination of residual undetectable disease to achieve a cure (9-10). In relapse treatment may revert to Induction, though there may be need to lower dosage depending on individual circumstance e.g., toxicity of previous therapy and level of morbidity (11). HSCT might be undertaken if Induction chemotherapy fails or a patient relapses despite Consolidation therapy(12). It may also be undertaken as first-line therapy alongside chemotherapy for patients with high-risk disease e.g., cytogenetic group, underlying myelodysplasia (MDS), or secondary and therapy-related AML (13).

Successful intervention is hampered by cytogenetic heterogeneity, toxicity (Box 1), multi-drug resistance (MDR), and age (14). Older patients (age >60) respond less well. In part this is due to the presence of co-morbidities but also greater association with MDS and secondary AML. In addition, clinicians have had a tendency to view therapy as palliative in older age groups, preferring to avoid toxicity, and studies have been

biased by exclusion of these groups. In many respects it has been this poor outlook for elderly and secondary AML that has driven new agents through phase I and II trials recently. Although the majority of patients under 60 years reach CR after intensive chemotherapy(15-16), relapse free survival (RFS) is uncommon. The 10-year overall (OS) and event free (EFS) survival for children/adolescents after Induction therapy is 55-65%. However, in adulthood only 20-40% of all patients gain disease-free survival (DFS) of >5 years from chemotherapy alone.

Radiation therapy for AML is generally used only if there is central nervous system involvement and no response to systemic and/or intrathecal chemotherapy (17). It may also be used in preparation for HSCT.

Box 1. Common side effects / consequences of chemothera.

Bone marrow suppression: Anaemia, Bleeding/Bruising, Infection
Hair loss
Nausea, Vomiting, Diarrhoea
Loss of taste
Mucosal ulcers (Mouth, Oesophagus)
Fatigue
Dry skin and brittle nails
Headaches, dizziness
Peripheral neuropathy (usually non-reversible)
Loss of hearing
Increased risk of secondary cancer
Loss of fertility, increased risk of foetal developmental abnormalities

Table 1. Induction Protocols with Conventional Chemotherapies

Principle	Agent / Study	Comment	Refs
Standardardising cytotoxic therapy	Childhood (under 18 years of age) Mitoxantrone or Daunorubicin in combination with Cytarabine and Etoposide.	The AML 12 trial showed a 10-year EFS of 54%, OS of 63% and relapse of 35% with no difference in CR between Mitoxantrone and Daunorubicin. Whilst Mitoxantrone showed a small benefit in a lower relapse rate this did not confer any OS advantage. The trial also showed no benefit of 5 over 4 cycles of treatment.	18-21
	Adults (agse 18 years and above) An Anthracycline for 3 days (Either: Daunorubicin, Idarubcin, or Mitoxantrone), in combination with 7 days of Cytarabine.	No other intervention shown to be better. **Younger Adults - 18-60yrs:** Treatment should be started immediately after diagnosis CR in 60-80% **Older patients - 60-74yrs:** CR in 50%, however with adverse cytogentics (Chapter 2) CR drops to 30% and Overall survival (OS) 5%. Timing of starting therapy in older patients should be individualized and based on comorbidities. **Patients >75yrs:** An alternative should be sought. Low dose Cytarabine is associated with longer survival in cytogenetically normal and mutated NMP1 (Chapter 2) case.	22-25 26-28
Comparative trials of Anthracyclines	Randomised studies have compared Daunorubicin with Idarubicin, Aclarubicin, Amsacrine, and Mitoxane.	No agent appears to be superior to Daunorubicin with respect to OS. They are equitoxic in older patients (>60years).	29-32 33-35
High vs Low Dose	High Dose Cytarabine (HiDAC)	No CR advantage of HiDAC over low dose. Not recommend as toxicity increased. HOVON/SAKK/AMLCG studies suggest Daunorubicin can be dose intensified in up to age 65 yrs with improved CR and survival.	36-41 41
Additional cytotoxic agents or Modulators of Multidrug Resistance	Thioguanine, Etoposide, Fludarabine, Topotecan	No increase in response rate.	42-47
Priming with growth factors to sensitize leukaemic cells	HOVON and SAKK studies AMLCG study ALFA study	Priming with G-CSF significantly increased DFS and OS respectively. Did not show impact on OS. GM-CSF increased the CR but no effect on OS.	48-51

'Old dogs, new tricks'

The focus of chemotherapy clinical trials research over the last 15 years has been to identify:

i. The most appropriate dose and frequency of administration,
ii. The efficacy of combination therapies,
iii. Whether certain agents are better than others by direct comparison,
iv. New formulations of established drugs (e.g., Liposomal drugs), and
iv. The value of adjunct therapies - facilitating effectiveness of chemotherapy and/or combating MDR.

Tables 1 (Induction) and **Table 2** (Consolidation) are a synthesis of trial literature demonstrating the development of chemotherapies over the last decade and a half, with reference to differences between age groups, and to CR and OS. Various Consolidation strategies have been evaluated including intensive conventional chemotherapy, prolonged maintenance treatment, and high-dose therapy followed by autologous or allogeneic HSCT.

The encapsulation of drugs in liposomes has led to new ways of more effectively delivering chemotherapy with a reduction in toxic side effects. Liposomal Daunorubicin has been shown in a Phase III trial to be as effective as normal Daunorubicin but better tolerated (60). The agent CPX-351 is a liposmal fixed combination of Daunorubicin and Cytarabine. Recent Phase I/II trials suggest CPX-351 to have an acceptible safety profile for use in older and previously untreated patients (61,62). Similarly, Elacytarabine, a derivative of Cytarabine but one that inhibits both DNA and RNA synthesis, has been demonstrated to be efficacious at least to the same degree as other agents, but with less toxicity in recent Phase II trials of patients in relapse requiring salvage therapy (63).

Given poor outcomes despite major advances in understanding best use of conventional chemotherapeutic agents, the need to develop novel therapies with different anti-leukaemic mechanisms is paramount. New agents entering the clinical arena include:

New Molecular Targeted Therapies

i. Monoclonal antibodies,
ii. Tyrosine kinase, and farnesyltransferase inhibitors,
iii. Cell growth blockers,
iv. Immunotherapies,
v. MDR1 inhibitors, and
vi. Peptide vaccines.

Table 2. Consolidation Chemotherapy.

Principle	Agent	Comment	Ref
High Dose Cytarabine (HiDAC)	Cytarabine	Young Adults - 18-60yrs The CALGB study showed HiDAC to be superior to lower doses - but, restricted in success to Core Binding Fusion-Gene AML (Chapter 2) and to a lesser degree Cytogenetically Normal AML. Other cytogenetic abnormalities are not affected by HiDA.	52 53
Comparative trials with HiDAC		Prolonged consolidation / multi-agent chemotherapy no better than HiDAC.	54-56
Prolongation of Maintenance therapy		No benefit in remission duration or OS compared to autologous HSCT in non-APL AM.	See HSCT below
Older Patients 60yrs or more. Consolidation therapy trials	 Cytarabine vs Cytarabine + Mitoxantrone. Cytarabine + Anthracycline or Thioguanine 6 cycles of Daunorubicin or Idarubicin + Cytarabine vs 1 standard consolidation Combination dosing of Idarubicin and Etoposide	No clear recommendations can be given. CALGB study - found no differences. AMLCG92 trial - older patients benefited with longer remission - particlularly effective in AML1-ETO AML (Chapter 2). AML-12 study also showed benefit in Childhood of consolidation with Idarubicin and Thioguanine. French ALFA 9803 - 6 cycles gave superior DFS and OS AMLSG AML HD98B trial	 57 58 18 35 59

Table 3. Molecularly Targeted Therapy in Clinical Trials and Practice.

Class / Agent	Comment	Refs
Monoclonal Antibodies: Gemtuzumab - Ozogamicin	Gemtuzumab is a humanised anti-CD33 antibody, approved in older patients who are not considered candidates for other cytotoxic therapies. Remission rates in 15% to 35% of older patients who have relapsed. Addition to standard induction therapy in younger adults led to a 91% CR rate.	64-68
Lintuzumab (SGN-33) Bevacizumab	This trial (a study in combination with Cytarabine) was discontinued in 2011 as mid trial results showed no additional benefit. Bevacizumab is directed against the vascular endothelial growth factor receptor (VEGFR). It also promotes survival of multipotential haematopoietic stem cells. It was the first angiogenesis inhibitor approved in 2004 by the FDA. Following a phase II trial demonstrating 33% CR further studies are now warrented.	69
FLT3 Tyrosine kinase inhibitors: Midostaurin Lestaurtinib Sunitinib Tandutinib Semaxinib Sorafenib	Several FLT3-selective tyrosine kinase inhibitors have in vitro cytotoxicity to leukaemia cells. Pilot studies combining intensive Induction and Consolidation therapy with FLT3 inhibitors have shown promising response rates in patients with FLT3 mutations.	70-74
KIT Tyrosine kinase inhibitors: Imatinib Dasatinib Axitinib	Imatinib competitively binds to the ATP-binding site of the tyrosine kinase Bcr-Abl, and targets KIT. It is the choice of treatment in Chronic Myeloid Leukaemia. Unfortunately the response rate was only 11% in a phase II trial of KIT positive AML patients treated with low-dose Cytarabine (LDAC) and Imatinib. However, it was no more responsive than LDAC, showing that LDAC monotherapy was just as good a 'blanket' treatment in patients unselected for the KIT molecular marker. Similarly Axitinib, which also inhibits VEGFR (Monoclonals above), has entered phase II study but not demonstrated any clinical efficacy. Dasatinib has been studied in vitro and its potential clinical benefit remains to be demonstrated.	75, 76
mTOR kinase inhibitors: Rapamycin Everolimus Temsirolimus Deforolimus	mTOR is a complex protein and a central regulator of many signalling pathways controlling cell division, metabolism and angiogenesis. There is evidence that its effect is manifest through a P13K/Akt pathway that is heavily dysregulated in haematological malignancies. Following phase II demonstration of efficacy and tolerability but minimal value as monotherapies, several agents are now being studied in combination with conventional therapies e.g., AML-12 trial (see below).	77,78
Farnesyltransferase inhibitors: Tipifarnib Lonafarnib BMS-214662	These agents can be given orally. They are in the early stages of phase I/II study. Tipifarnib in combination with Etoposide has recently been shown to achieve 50% CR in very poor risk older patients, which is extremely encouraging. Lonafarnib has limited activity in older patients where focus was on treating secondary AML. Four out of 9 patients entered CR in a phase 1 study of BMS-214662; sufficient evidence to recommend a phase II trial.	79 80 81
Hypomethylating (nucleosidase analogue) agents: Azacitidine and Decitabine	These act by inhibition of ribonucleotide reductase and DNA polymerase, inducing apoptosis. Two demethylating agents, the Cytosine analogues Azacitidine and Decitabine, have been approved for the treatment of MDS (Chapter 1). Azacitidine prolonged OS compared with conventional care regimens in patients with intermediate- or high-risk MDS (of which 1/3rd had AML). 2-year OS was 50% with Azacitidine compared with 16% with conventional treatment regimens. Azacitidine has been approved for older patients with AML with 20% to 30% blasts. Decitabine (Dracogen) has recently been rejected by the Federal Drug Administration (FDA) for its 'unfavourable risk/benefit profile'.	82-86

Other Nucleosidase Analogues: Clofarabine Troxacitabine Sapacitabine	Clofarabine is a purine nucleoside analogue synthesized to combine the most favourable pharmakokinetic properties of Fludarabine and Cladribine. Phase II trials are required but an initial study (hampered by hepatotoxicity) showed a 44% OS and 21% CR in elderly patients who were otherwise unfit for intensive chemotherapy. After some success in small phase I and II studies a larger scale trial of Troxacitabine was terminated in 2006 after 6 months with only 10-15% of patients acheiving CR. Sapacitabine is now in phase III trial. It is an oral agent that may be particularly useful in untreated elderly patients, first relapse, or secondary AML following MDS treatment.	87-91
DNA alkylating agents: Laromustine (Cloretazine)	Laromustine was shown to have a CR of 35% compared to 19% for Cytarabine in a phase I study of patients with relapse or refactory disease. However the OS in both groups was the same.	92
Immunomodulators:	The agent Ceplene (Histamine) has been licensed for use with Interleukin-2 (IL-2) in Europe but not in the UK or USA. The FDA recently advised that the Phase III trial that demonstrated effacy for maintenance of remission be re-opened with a comparator arm of IL-2 monotherapy despite 5 previous studies showing IL-2 monotherapy to be ineffective.	93
Histone Deacetylators: Panobinostat Vorinostat Entinostat Mocetinostat Valproic Acid	Phase I and II studies have not shown these agents to have any clinical activity as monotherapies. Further research is needed to determine whether they have any synergistic effects with other cytotoxics. A phase II clinical trial of Azacetidine, Valproic acid and All Trans Retinoic Acid has recently demonstrated improved CRs (20-30%), albeit still short OS in patients with high-risk acute myeloid leukemia or myelodysplastic syndrome.	94,95
Multidrug Resistance (MDR) Modulators: Valspodar Zosuquidar	Several MDR modulators have been studied. All are lipophilic and include the immunosuppressant Cyclosporine. Valspodar is potent Cyclosporine derivative without immunosuppresant effect or the renal toxicity of Cyclosporine. Unfortunately the outcome of the phase III AML 14 trial showed a poorer outcome in the Valspodar treated group. Phase I studies of Zosuquidar (blocks drug clearance through P-gp transporters) have shown it to be tolerable and have significant effect on clearance of Cytarabine and Daunorubicin in vitro. It now needs to enter phase II trials to establish whether it has any clinical effectivenss as an adjuvant therapy.	96 97
Vaccines:	The basis of peptide vaccination is the observation that leukaemia cells (LCs) can act as antigen-presenting cells (APCs). Through HLA class I and II pathways peptides are presented to CD4+ helper and CD8+ cytotoxic T lymphocytes. Peptides can be introduced in to LCs differentiated from normal cells by surface markers (Chapter 3). The LCs then acting as APCs present a peptide to T cells in a more efficient manner than AML blasts might do alone. These lymphocytes become activated and proliferate. Once such primed T cells encounter the same epitope peptide on the surface of an AML blast, the malignant cell can be lysed. These have looked at the impact of Proteinase 3, RHAMM and WT1 interventions. Table 4 is devoted to this area given the number of specific trials. The WIN Study is a Phase II trial. Recruitment closes in July 2012. This is looking at responses to infuence expression of gene fusion associated with WT1 .	98
Chromosome 5 abnormalities:	The Len5 study closed to recruitment in Nov 2011 and is on-going. It is exploring the efficacy of Lanalidomide (Revlimid) in AML subgroups associated with Chromosome 5 abnormalities.	

Table 4. Peptide Vaccination in AML.

Antigen	Peptide sequence/amount	Patients included in the trial per entity	Clinical status before vaccination	Ref
Proteinase 3	Pos. 169-177 VLQELNVTV (designated 'PR1') 0.25, 0.5 or 1.0 mg sc. q3wks × 3	42 AML, 13 CML, 11 MDS	53 active disease, 13 CR	99,100
RHAMM	Pos. 165-173 ILSLELMKL (designated 'R3') 300 ¿g sc. q2wks × 4	2 AML, 4 MDS, 4 MM	Limited tumour load or MRD	101
	R3 peptide 300 ¿g sc. q2wks × 4	6 B-CLL	Early stage (Binet A)	102
	R3 peptide 1000 ¿g q2wks × 4	1 AML, 5 MDS, 3 MM	Limited tumour load or MRD	103
WT1	Pos. 126-134 RMFPNAPYL 0.2 mg sc. q2wks × 4 then q28d up to 23 times (until progression)	24 AML, 2 MDS	18/26 active disease, 8 CR	104,105
	Pos. 235-243: CMTWNQMNL with modification CYTWNQMNL 0.3, 1.0 or 3 mg id. q2wks × 3	12 AML, totally 26 (including 2 breast cancer, 10 lung cancer and 2 MDS)	12 CR	106
WT1 and proteinase 3	PR1 peptide 0.5 mg WT1. Pos. 126-134 0.2 mg sc. 1×	5 AML, 1 CML, 2 MDS	1 RARS, 2 RA, 4 CR (AML) and 1 (CML) CP	107,108
	PR3 and WT1 q2wks × 6	3 AML, 1 MDS	<30% blasts in the BM	109

Adapted from Scmitt M et al (98).

Table 3 and **4** outline trials in this area. In the majority of cases these trials have small numbers of patients and/or have selected for more complex disease and non-responders.

On the whole the inhibition of deregulated transcriptional activity consequent on gene mutations has not led to therapeutic innovation; the exception to this is all-*trans*retinoic acid and arsenic trioxide in APL.Inhibition of tyrosine kinase activity, nucleoside analogues, and monoclonal targeting of the antigen CD33 have demonstrated some success but none of these agents are superior to the combination Induction and Consolidation highlighted in **Table 1** and **2**.

Combinations of novel and current therapies are currently being explored in multi-faceted/multi-centred trials. The AML-17 (recruitment from 2002 to 2014) is one such phase III study looking at:

i. The best dose of Daunorubicin,
ii. CEP-701, a new FTL3 inhibitor,
iii. Everolimus (Afinitor) a signal transduction inhibitor that blocks the signalling protein mTOR,
iv. The comparison of 2 chemotherapy treatments before HSCT,
v. The comparison of 1 with 2 or more cycles of chemotherapy, and
vi. The role of Arsenic Trioxide in non-APL AML.

And in addition:
i. Clofarabine, a nucleoside analogue (see above), is being compared with other chemotherapies having been shown to have fewer side effects but similar efficacy to Fludarabine in the treatment for older patients considered unsuitable for induction chemotherapy, and
ii. Studies will continue to look at the role of Gemtuzumab.

There does not appear to be a revolutionary step change in drug therapy on the horizon in the management of AML. Attention is focused on synergistic effects of combining conventional with novel targeted agents. Though targeting of leukaemic stem cells (LSC) by, for example, receptor specific small molecules and peptide vaccines would appear a reasonable approach, the similarities between LSC and normal stem cells is also a challenge. Currently the targeting of LSCs remains relatively non-selective and requires simultaneous interventions.

Haematopoeitic stem cell transplantation

The developmental milestones in stem cell therapy (SCT) from the 1950s from preclinical trials to the successful application in human transplantation in the late 1970s are shown in **Table 5**. These laid the foundation for many areas of stem cell research, as well as current HSCT practices.

Four sources of HSCT are available and each has its pros and cons (**Table 6**). The vast majority of clinical trials have been in allogenic and autologous stem cell transplants (SCT). Despite our learning of how best to use these therapies the challenges remain better techniques for cryopreservation,

Table 5. Developmental milestones in HSCT.

Year	Development of Haematopoietic Stem Cell Transplantation	Challenges
1949	Spleen shielding experiment of Jacobson.	Limited knowledge of radiation in immune-suppression
1957	First human twin transplants for leukaemia.	Relapse
1962	Successful allogeneic transplants in dogs	Understanding of human histo-compatibility
1968	First successful allogeneic transplants in humans.	Graft-vs-Host Disease (GVHD), limited understanding of details of human histocompatibility, lack of experience with the use of immunosuppressive drugs, and shortcomings in supportive care techniques
1977	Successful application of autologous marrow transplantation	Lack of genetic markers, poor cryopreservation technology
1979	Encouraging results in patients with acute myeloid leukaemia transplanted in first remission	GVHD and complications, Relapse, toxicity, and limited donor compatibility and availability.

Table 6. Pros, Cons and Challenges of the different types of HSCT.

TYPE OF SCT	PROS	CONS	CHALLENGES
Allogeneic SCT	GVL effect represents one of the most powerful anti-leukaemia treatments.	GVHD, Treatment-related mortality (TRM)	Improving current sources of transplantation and incorporating novel therapies to mitigate TRM
Autologous SCT	Immunologic compatibility between infused haematopoietic stem cell.	Absence of GVL which is crucial to achieving good outcome in SCT, shorter DFS	Contaminated sample, elucidating autologous stem cell transplantation in conjunction with gene therapy
Umbilical Cord Blood	Greater availability, increase in eligible donors and decreased incidence of GVHD	Decreased numbers of stem cells, increase graft failure and mortality.	Overcoming cell dose limitation.
Induced pluri-potent stem cells (Chapter 2)	Prospects to generate SC uncontaminated for autograft without ethical complications	Genomic instability, tumour formation, and the lengthy time requirements needed to obtain these cells via retrovirus development	Locating pluripotent stem cell sources without the need for reprogramming protein integration

identification and classification of genetic markers, and understanding the influence of SNPs (3), non-HLA genetics, and cytokine genes (110-112).

In patients with favourable- and intermediate-risk cytogenetics, autologous HSCT is an alternative Consolidation option to chemotherapy. It is not recommended in cases with high-risk cytogenetics (113-115). There is no evidence that this approach gives a better outcome in general, however it may be of advantage in cytogenetically normal and tandem repeat subsets of AML (116). The lowest relapse rates are observed following Consolidation with allogeneic HSCT. The benefit is in part attributable to a potent graft-versus-leukaemia (GVL) effect (117). Meta-analyses of clinical trials comparing allogeneic HSCT versus Consolidation chemotherapies after first CR show a significant improvement in OS in intermediate- and high-risk AML (118-120). **Table 7** shows data from several studies in the 1990s. The DFS following Consolidation is in general superior for allogenic vs autologous HSCT, and for HSCT vs chemotherapy. However OS rates were not significantly different in a number of these studies. Data from the 2000s in childhood disease is more compelling for favourable outcome of DFS and OS after allogenic HSCT (**Table 8**).

It is important to consider the risks of treatment-related mortality (TRM). These range between 15-50% and may

Table 7. Comparative Disease Free Survival following Allogeneic HSCT, Autologous HSCT, and Chemotherapy for AML patients in first remission

Study (date)	Treatment	No of pts	DFS	P value	OS	P value	Relapse	P value	Ref
Franc e (1989)	Allo Auto Chemotherapy	20 12 20	66% 41% 16%	<0.004			18% 50% 83%	<0.0002	121
Netherlands (1990)	Allo Auto	23 32	51% 35%	NS	66% 37%	0.05	34% 60%	0.03	122
EORTC/ CIMEMA (1996)	Allo Auto Chemotherapy	168 128 126	55% 48% 30%	significant	59% 66% 46%	NR	27% 41% 57%	NR	123
GOELAM (1997)	Allo Auto Chemotherapy	88 86 78	44% 44% 40%	NS	53% 50% 55%	NS			124
US Intergroup (1998)	Allo Auto Chemotherapy Allo Auto	113 116 117 92 63	43% 34% 34% 47% 48%	NS NR	46% 43% 52% 45% 55%	0.04 0.05 NR	29% 48% 62%		125
MRC (1998)	Auto Chemotherapy	190 191	53% 40%	0.04	57% 45%	0.2	37% 58%	<0.01	126

Allo: Allogeneic, Auto: Autologous, NS: not significant, NR: not reported, DFS: Disease Free Survival, OS: Overall Survival. Adapted from Blume and Thomas (127).

Table 8. Comparative outcome data in Childhood Trials of Allogeneic HSCT, Autologous HSCT, or Chemotherapy for AML

Trial identification	Risk groups	No of Patients	DFS (%)	OS (%)	Reference
AML88	High risk	17 allo 31 auto	74 (8 years) 74		128
CCG2891	Not stated	177 auto 179 chemo 181 allo P-value	42 (8 years) 47 55 0.01	48 (8 years) 53 60 0.05	129
MRC10	Low 28%, Medium 52%, High 20%	85 donor 230 no donor P-value 50 auto 50 no therapy P-value	68 46 0.02	70 (7 years) 60 0.10 70 59 0.20	130
CCG 251, 213, 2861, 2891, 2941	Not stated	373 allo 217 auto 688 chemo P-value (allo vs chemo)	47 (8 years) 42 34 0.004	54 (8 years) 49 42 0.06	131
AML BFM 98	High risk	58 donor 166 no donor P-value	47 (5 years) 41 0.40	55 (5 years) 54 0.16	132

Allo: Allogeneic, Auto: Autologous, DFS: Disease Free Survival, OS: Overall Survival. Adapted from Klingebiel et al (133).

out-weigh the benefits. These risks have been improved for older patients in particular by using reduced-intensity chemotherapy (RIC) regimes that are non-myeloablative. The outcomes are much more promising and comparable with younger cases (134,35),although relapse rates remain a challenge (136). Data available from the European Group for Blood and Marrow Transplantation (EBMT) and the Centre for International Blood and Marrow Transplantation Research (CIBMTR) demonstrates that RIC regimens result in comparable outcomes across the adult age range (**Table 9**) (137). As raised above for chemotherapy, data is difficult to interpret for older patients due to small patient numbers, heterogeneity, and selection bias. For these reasons prospective comparison of allogeneic HSCT from matched related and unrelated donors using RIC with conventional Consolidation therapy was launched in 2008 and as of December 2011 continues to recruit to this important clinical trial (ClinicalTrials.gov Identifier: NCT00766779). The use of RIC led to concern over graft versus host effects (**Table 6**) and has prompted research into immune-suppression studies to modulate GVL and GVH reactions (138). Finally, research continues in to the benefit and most appropriate regimens using umbilical cord blood (**Table 6**) (139).

Novel pathways and potential future agents

Several pathways and novel mechanisms of intervention are the focus of attention in current AML research.

AKT Inhibitors

As raised in **Table 3** mTOR is a kinase involved in regulation of cell growth and proliferation. Signalling depends on its interaction through the PI3/Akt pathway (140). Both PI3K and Akt are considered to be protooncogenes. Increased membrane expression of Akt is important in intiating malignancy. It also appears to confer resistance to apoptosis through the mitogen-activated protein kinase (MAPK) pathway (141). As well as targeting mTOR (**Table 3**), Akt and MAPK inhibitors may represent new classes of drug. Perifosine is an Akt inhibitor and has shown preclinical activity against haematologic malignancies (142). Phase I and II trials have been conducted in patients with solid tumours but not in leukaemias (143). Alkylphosphocholines are lipophyillic drugs that have also been shown to modulate signal transduction by their interaction with c-myc, PI3-Akt, and MAPK pathways. Erufosine, an alkylphosphocholine, has anti-leukaemic properties that warrant further exploration (144).

RPRDX2

Studies have demonstrated that AML blasts exhibit significant lower levels of Histone H3 acetylation (H3Ac) compared to CD34+ progenitor cells. As a consequence it is suggested that a number of genes are epigenetically silenced or diminished in AML. Agrawal Singh et al (145) recently showed that Peroxiredoxin 2 (PRDX2) is a novel potential tumour suppressor gene in AML. H3Ac was decreased at the PRDX2 gene promoter in AML, and correlated with low mRNA and protein expression. Low protein expression of the antioxidant*PRDX2*gene was clinically associated with poor prognosis in AML. They identified PRDX2 acts as an inhibitor of myeloid cell growth by reducing levels of reactive oxygen species (ROS) generated

Table 9. Comparison of EBMT and CIBMTR data in Elderly Patients

	n	Median age	RIC conditioning	OS	Relapse Rate	
CIBMTR*						
40-54	208	78% (p=.07)	44% (p=0.06)	33% (p=0.87)	25% (p=0.26)	
55-60	146	68%	50%	34%	22%	
60-65	126	69%	34%	37%	32%	
>65	55	65%	36%	33%	34%	
	n	**Median Age**	**RIC conditioning**	**OS**	**Relapse rate**	**Non-Relapse Mortality**
EBMT**						
50-60	884	54 (50-60)	55% (p<0.01)	34% (p=0.23)	32% (p=0.02)	36% (p=0.39)
>60	449	63 (60-75)	78%	24%	41%	39%

*2 year estimates **4 year estimates. Adapted from Patel et al (137).

in response to cytokines. Taken together, epigenome-wide analyses of H3Ac in AML, led to the identification of PRDX2 as an epigenetically silenced growth suppressor suggesting a possible role of ROS in the malignant phenotype in AML. This may be a pathway to explore in the application of Histone Deacetylator agents (**Table 3**).

TET2 mutation

Over the last 2-3 years mutations of Ten-Eleven Translocation 2 (TET2), have been found in various myeloid malignancies. The gene is associated with DNA methylation, mutations leading to inhibition or reduction in appropriate myeloid cell differentiation (Chapter 2) and appears to be a prognostic biomarker in AML associated with intermediate-risk cytogenetics (146-153).

In a study last year by Weissmann et al (154) 131 somatic TET2 mutations were identified in 87/318 (27.4%) patients, and in 30% of cases of normal karyotype AML versus 19% of abnormal karyotype. Mutations of TET2 were concomitantly observed with mutations in NPM1, FLT3-ITD, FLT3-TKD, JAK2, RUNX1, CEBPA, CBL and KRAS (Chapter 2). Patients tended to be of older age, with higher haemoglobin level, higher neutrophil and monocyte counts, and lower platelet count. Similar mutational associations were identified by Chou et al (155). Survival analyses (restricted to the normal karyotype population (n=165)) in Weissmann' study showed inferior EFS in the presence of TET2 mutations.

In two other studies, one retrospective (156) the other prospective (157) the presence of TET2 mutations did not appear to influence CR or OS after standard therapy. There has also been one clinical study in higher risk MDS and AML with low blast count, where TET2 status was observed to be a genetic predictor of response to Azacitidine, independently of karyotype (141). Further clinical studies with such hypomethylating agents are warranted.

DNMT3A mutation

DNMT3A mutations are observed in up to 22% of AML patients and appear more prevalent in the intermediate-risk groups, and especially of normal karyotype (158). The mutations are strongly associated with poor prognosis (159-161), and like TET2 are associated with decreased DNA methylation and promotion of cell differentiation. DNMT3A forms a complex with transcription factors like histone methyltransferase and histone deacetylase (162,163). Novel DNA methyltransferase and histone deacetylase inhibitors can reverse the methylomic phenotype of myeloid blasts (**Table 3** Nucleosidase and Histone Deacetylase Drugs).During therapy, early platelet response and demethylation of the FZD9, ALOX12, HPN, and CALCA genes were associated with clinical response. Epigenetic modulation deserves prospective comparisons with conventional care in patients with high-risk AML, at least in those presenting previously untreated disease and low blast count.

Trial methodology

Pre-clinical

In vitro studies of cultured native AML cell lines and blasts have remarkably contributed to our current understanding on the pathogenesis of AML (**Table 10**). Well-characterised serum-free in vitro conditions are now used in experimental studies of AML, facilitating comparisons between different experiments. Assays for characterisation of AML progenitor subsets such as suspension cultures, colony assays, long-term in vitro culture, xenotransplantation in immunocompromised mice, as well as, AML cell lines as experimental models have been used to increase our knowledge on pathogenesis of AML (164). Furthermore, biomarker studies suggest that the in vitro growth characteristics of AML blasts assayed by short-term culture of the total native populations can be used as a predictor of prognosis after intensive chemotherapy. In vitro assays may be used for more accurate identification of prognostic parameters and for creation of a basis for the development of simplified laboratory techniques suitable for routine evaluation of patients undergoing risk-adapted therapy (164).

Clinical

Drug development processes are lengthy and costly. While the phase I-III sequence of clinical drug testing has remained intact for decades, it appears inherently inefficient and the high frequency of false-positive results obtained in phase II studies constitutes a significant scientific concern (175-180). The sequential trial scheme puts major emphasis on such studies because they typically inform the decision to proceed to a phase III evaluation (175). Strategies to mitigate shortcomings caused by lack of control groups, patient heterogeneity, selection bias, and choice of end points and strategies for streamlining trial design have been suggested. Such enhancements would among others encompass larger phase II studies, inclusion of (preferably randomised) controls, consideration of integrated phase 2/3 studies, accounting for patient heterogeneity even in small randomised studies, provision of information about the number of patients available for study vs. those actually treated, and avoidance of unvalidated alternate endpoints and premature publication (**Table 11**) (175).

Phase I trials often provide novel agents to patients with relapsed and refractory disease (181). It has been argued that

Table 10. Experimental models for the study of AML cell proliferation.

Experimental model	Description of the experimental procedure	Dominating phenotype of proliferating cells	Comments
Short-time suspension culture with 3H-thymidine incorporation	The total population of native AML cells cultured for six to seven days before nuclear radioactivity is determined (165).	Probably clonogenic cells of the phenotype CD34- (167-169).	Reflects an enrichment of colony-forming cells, and assays the response of a subset of AML cells able to proliferate after one week of in vitro culture (168. Regarded as more sensitive than the colony (170).
Primary colony formation	Native AML cells seeded directly in a colony-forming assay (166-168).	Fluorouracil-sensitive CD34- cells (171).	Depending on the culture conditions (medium alone or addition of exogenous cytokines), colonies can be differentiated into small abnormal clusters of uniform morphology, blast-like/monocytic and erythroid colonies (172). Colony-forming cells are a minority among native AML cells (usually <3%) (169).
Long-term suspension culture	Culture of AML cells in suspension culture for two to eight weeks before the number of colony-forming cells in the population is estimated (166, 168).	CD34+CD71-CD90-HLA-DR- for most patients; in exceptional patients CD34- (166, 172).	The frequency of suspension culture initiating cells (SCIC) is usually lower than the frequency of primary colony-forming cells (168).
Cobblestone-area forming cells	Suspension culture of AML cells on a stromal layer in the presence of exogenous cytokines; the number of colonies with cobblestone morphology is determined after several weeks of culture (170).	Fluorouracil-resistant progenitors (170).	The most primitive cobblestone-area forming cells (week 6) are less sensitive to Fluorouracil than less primitive (week 2) cells The frequency of these progenitors seems comparable to the suspension-culture initiating cells (170).
SCID mouse repopulating cells	A xenotransplant model with engraftment of AML cells in combined immunodeficient mice (172).	Usually CD34+CD71-CD90-HLA-DR-, in exceptional patients CD34- cells. The cells are Fluorouracil resistant (170).	The most effective SCID-repopulating cells constitute a small minority of CD34+CD38-HLA-DR- cells among native AML blasts The number of cells needed for engraftment varies between patients (173-174).

Adapted from Bruserud Ø et al. (164).

noncytotoxic, molecularly targeted agents have not been very successful in this setting. Thus, signals of their true biologic efficacy may be missed and consequently potentially useful agents seem fail to demonstrate a signal of efficacy in the phase I setting. It has been proposed that at least some of these compounds should be considered instead in trials to prolong response duration (181).

Phase II trials in AML are usually small-scale and may give misleading efficacy signs (175, 166). Due to the heterogeneity of the disease, subset analyses based at least on age, performance status, cytogenetics, and molecular features are necessary (175). However, these are meaningless when the total group includes a small number of patients (181). Consequently, there is increasing support for randomised phase II trials strategies planned to quickly compare new treatments with existing standards using as few patients as possible and to proceed only with those that meet predetermined efficacy benchmarks (181).

Phase III trials in AML are often slow, expensive to complete and, regrettably, often resulting in minor improvements. It has also become increasingly difficult to determine a feasible control group for new phase 3 trials due to the large number of molecularly and clinically defined subgroups. Further enhancements in the molecular characterisation of AML could allow the identification of more homogeneous treatment cohorts and tailored therapeutics (181).

Translational strategies to accelerate drug development

- **Focusing on development of existing drugs in addition to searching for new ones.** Due to the heterogeneous pathogenesis and molecular genetics of AML, tailored, personalised treatment based on the specific biologic features of the leukemic cells should be the objective. This also indicates that combination therapy is likely to remain superior to any single compound (181). One interesting suggestion is to use the gene-expression signature generated from drugs that effectively ablate LSCs to study publicly available databases for other similar signatures (182). In the case of "off-patent agents" , this could possibly al-

Table 11. Suggestions for improvements of clinical trial designs in AML.

Problem	Possible solution
Cost and inefficiency of oncology drug development, especially in phase II/III trials	More attention should be given to the conduct of phase II trials to minimise the risk of overly optimistic reporting of results and to limit the number of subsequent negative phase III trials. Creation of better strategies for identification of phase II trial characteristic that predict a positive phase III study. Focus on characteristics in trial design may help optimise drug development and minimise the resources expended on drugs that will likely fail in later stages of drug testing.
High false-negative and false-positive rates	Increase in study sizes. To properly interpret the results of phase II studies, scientific reports should, specify the false-positive and false-negative errors associated with number of patients treated; furthermore, for studies that use the experience of previously untreated or differently treated patients as a basis for comparison, the number of such patients along with their distribution of clinical characteristics should be provided.
Ill-defined historical control group	Explicit description of the control group (number of patients, type of study, diagnoses, treatment); adjustments for sampling variation and differences in case mix.
Lack of control group makes it difficult to estimate how good results truly are	Use of explicitly described historical or concurrent control group; randomization, including multi-arm, multi-stage designs.
Handling patient heterogeneity	Stratified trial; statistical adjustment (multivariate analysis).
General is ability of treatment results, effect modification	Explicit description of inclusion/exclusion criteria, provision of information about total number of patients available for study vs. those actually treated.
Choice of surrogate endpoint that does not predict clinical benefit	Use of validated surrogates; validation of alternative endpoints before use.
Delay in activation of phase III trial	Integrated phase II/III trial design; streamlining of internal and external groups and processes. Adaptive trial design.
Bias through early publication	Allowance of adequate follow-up time between completion of study accrual and publication; introduction of journal policies to discourage too early publication.

Adapted from Walter RB et al. (175).

low existing agents with well-identified clinical profiles and easy availability, to rapidly lead into AML treatments (181).

- **Increasing participation in clinical trials.** Less than 5% of adult cancer patients in US participate in clinical trials, contrary to 60% of paediatric cancer patients (181,183). Recently, a French survey reported that 25% of AML patients among 1066 adults with AML were enrolled in clinical trials (181,184). Physician and patient education about clinical trials should be enhanced and collaboration between academic centers and cooperative groups should be improved (181). Increasing accrual in clinical trials is vital, as there the traditional phase I-III drug-development paradigm seems ineffective in this disease (175,181).

- **Improving of safety and efficiency.** Implementing biomarkers in clinical trials may improve decision-making in drug development process (185). Biomarkers predicting therapeutic response enable the selection of patients most likely to have positive treatment outcomes with a particular oncologic therapy. Predictive pharmacogenomic biomarkers, enabling selective treatment, are likely to become increasingly common in future therapies (186). Biomarkers predicting the safety of a compound are highly valuable for preclinical testing, or early clinical studies. Microdosing studies could be used for improving safety when evaluating drug candidates at early stage development. Adaptive trial designs in AML studies could improve safety and efficacy by providing opportunities to make changes to a study in response to accumulating data whilst maintaining the trial's integrity and validity.

Conclusion

AML is characterised by a multitude of chromosomal abnormalities and gene mutations, which translate to marked differences in responses and survival following chemotherapy, radiotherapy and HSCT. These chromosomal and genetic abnormalities make the treatment of AML challenging. The limit of acceptable toxicity for standard chemotherapeutic drugs used in AML therapy has been reached. A detailed under-

standing of the molecular changes associated with chromosomal and genetic abnormalities is necessary to pilot new therapy design. Although several deregulated proteins and genes have been identified, their diversity among AML patients have made it difficult to identify a single substance that can hit these diverse targets . New agents have shown promise but there remains a huge need to be met for effective and targeted therapies to be successful.

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