Prognostic factors and therapeutic options for relapsed or refractory acute myeloid leukemia

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A B S T R A C T

Background and Objectives. Considerable progress has been made in the treatment of acute myeloid leukemia (AML); however, current therapeutic results are still unsatisfactory in untreated patients and poorer in those with primary refractory or relapsed disease. The biological and clinical characteristics of relapsed AML are analyzed here.

Design and Methods. Most relevant literature in English language on relapsed AML from 1990 to 2004 was considered paying particular attention to the heterogeneity of the disease and prognostic factors at the time of relapse; therapeutic results in terms of second complete remission (CR) with conventional chemotherapy, stem cell transplantation and new agents are also summarized.

Results. Molecular relapse is a current indication for treatment of acute promyelocytic leukemia (APL); however, data are emerging for the treatment of molecular relapse in AML other than APL, such as AML with t(8;21) and AML with inv(16). Age, duration of first remission and cytogenetics are the most relevant prognostic factors in relapsed AML. Promising therapeutic results have been reported for the antiCD33 monoclonal antibody conjugated with calicheamicin and the new nucleoside analog clofarabine; preliminary studies indicate that FLT3, farnesyl-transferase and bcl-2 inhibitors are active in relapsed AML.

Conclusions. All relapsed elderly patients and young adults with CR1 lasting for less than 12 months are ideal candidates for experimental therapies. Efficiently conducted phase II randomized trials are needed in order to achieve relevant information to be translated into phase III trials.

Key words: acute myeloid leukemia, relapse, prognostic factors, therapeutic options.

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Acute myeloid leukemia (AML) is a malignant blood disorder characterized by blocked or impaired differentiation of hematopoietic stem cells, resulting in abnormal accumulation of immature precursors and suppression of growth and maturation of normal hematopoiesis.[1] Over the past years, the application of novel cytogenetic and molecular techniques has markedly improved our knowledge on the pathophysiology of the disease, resulting in new potential therapies.[2-4] Notwithstanding this, current therapeutic results are still unsatisfactory, particularly in patients of advanced age and/or in those with primary refractory or relapsed disease, due to difficulties in translating new pathogenetic insights from the bench to bedside.[5-12] In particular, recurrence of AML after a complete remission (CR) has been achieved still represents a major obstacle to overcome when cure is the objective of the treatment.[7-12] In this review, we will focus on the most relevant biological and clinical aspects of relapsed AML, including prognostic factors, current therapeutic results with conventional chemotherapy and stem cell transplantation, and new therapeutic strategies based on the selective inhibition of pathways involved in leukemogenesis and/or the immunologic targeting of leukemic cells.

Clinical presentation of relapse in AML

Three patterns of relapse can be distinguished in AML, i.e. hematologic relapse, extramedullary relapse and molecular relapse. Hematologic relapse is defined as the reappearance of blast cells to constitute more than 5% of the cells in the bone marrow and is generally considered as an absolute indication to treatment.[13] It is

important to quantify the level of leukemic bone marrow infiltration exactly, because this knowledge may influence the therapeutic decision as will be discussed in the section on stem cell transplantations. Extramedullary (EMD) relapse can occur either in isolation, raising the need for local radiotherapy followed by any kind of consolidation, or in association with hematologic relapse.[14,15] It more often occurs in core-binding-factor (CBF) AML, i.e. AML with t(8;21) and AML with inv16, presenting as granulocytic sarcoma.[16] Less frequently, the central nervous system (CNS) may be involved with the typical picture of meningeal leukemia. A particularly intriguing finding is the occurrence of EMD relapse in patients with acute promyelocytic leukemia (APL) treated with all-trans retinoic acid (ATRA) in combination or not with chemotherapy, the most frequently involved sites being the CNS, skin and middle ear.[17,18] However, a large study by Specchia *et al.* did clearly show that EMD relapse is infrequently observed in APL and that patients receiving ATRA plus chemotherapy (AIDA protocol) have a similar risk of undergoing relapse with EMD involvement as do patients treated with chemotherapy alone.[19] Moreover, data from this study pointed to a different pattern of EMD localization in the two cohorts, with a higher prevalence of CNS involvement at relapse in patients receiving ATRA.[19] Thus, prophylaxis with inthrathecal chemotherapy should be considered for high risk APL patients, defined as those having a white blood count over 10×10^6/L at diagnosis.[20]

At the moment the detection of molecular relapse results in the need for treatment only in APL patients.[13,21,22] Early work from the GIMEMA group clearly demonstrated that APL patients treated in molecular relapse fare significantly better than those treated in hematologic relapse.[23,24] Such an approach, now accepted world wide, has significantly improved the overall outcome of patients with APL[24] and represents a paradigm for the concept of molecular relapse in AML.[21] An intriguing question is whether we can extend the APL model to other AML subtypes. In CBF-AML the cytogenetic abnormality results in the formation of hybrid fusion genes, i.e. AML1/ETO for t(8;21) and CBFβ/MY11 for inv(16)/t(16;16), which can be quantified in CR patients.[26] While previous studies[16] claimed that persistent positivity for the AML1/ETO hybrid gene in AML with t(8;21) had no or only poor prognostic relevance, recent data based on quantification of fusion transcripts in AML1-ETO+ AML or CBFβMYH11+ AML show these are strongly predictive of prognosis and can be considered in the selection of post-remission therapy or in decisions of whether to treat patients with a progressive increase in the amount of hybrid transcripts.[27-29] In a multicenter study of 51 patients with t(8;21) in first or second CR, all samples were tested by two different reverse transcription polymerase chain reaction (RT-PCR) techniques (a nested technique and a one step technique with a sensitivity of 10^{-6} and 10^{-5}, respectively). Samples from 14 potentially cured patients (median follow-up 112 months) were taken at least twice and all were PCR negative by both techniques. In addition, samples from 37 patients were prospectively taken after the patients had achieved CR1 and/or CR2, before consolidation treatment, and every 3 to 6 months after completion of therapy. Of interest, patients who converted to PCR negativity after achieving CR as well as for those who became PCR-negative with the one step technique before intensive consolidation treatment were observed to have a better prognosis. The one-order lower sensitivity of one-step PCR may be the explanation for its better clinical usefulness.[30]

Finally, for patients in whom specific molecular abnormalities are not detectable, monitoring Wilms' tumor 1 gene (*WT1*) expression has been shown to add relevant prognostic information that may be useful for treating molecular relapse in patients in hematologic remission.[31-33]

Prognostic factors for relapsed AML

Different clinical and prognostic parameters have a major role in predicting prognosis in newly diagnosed AML patients.[34] In particular, age, cytogenetics and early blast clearance have been found to be significantly related to CR rate and survival, better results being achievable in young adult patients with favorable cytogenetics.[35] As far as cytogenetic abnormalities are concerned, two substantially overlapping classification systems have been proposed by the United Kingdom Medical Research Council (MRC) Cooperative group[36] and the South West Oncology Group from the United States.[37] Accordingly, three groups can be distinguished: (i) a better prognosis group including those with t(8;21), inv(16) and t(16;16), accounting for 10-15% of patients and more commonly occurring below the age of 60 years; (ii) a group with an unfavorable prognosis, more frequently comprising elderly patients, with monosomies or long arm deletions of chromosomes 5 and 7 and abnormalities involving 3 or more chromosomes (complex karyotype); (iii) an intermediate group accounting for 50-60% of cases including normal karyotypes and all other aberrations. While the prognostic relevance of karyotype in primary AML is well established and universally accepted, the value of cytogenetics at diagnosis for relapsed patients is not so well defined.[38] Notwithstanding, in univariate analysis different authors have demonstrated that relapsing patients with adverse cytogenetics at diagnosis have a poorer outcome than those within the favorable or intermediate subset.[39-42] However, results

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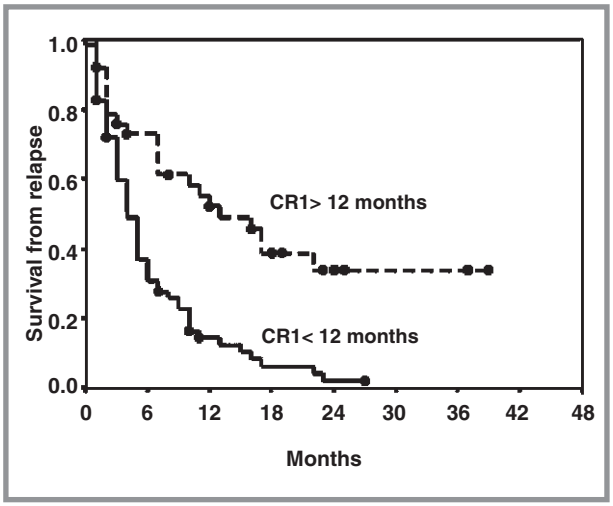

Figure 1. Overall survival from a series of 101 patients observed at our institution with relapsed AML according to CR1 duration, i.e. > or < than 12 months (p = 0.0001).

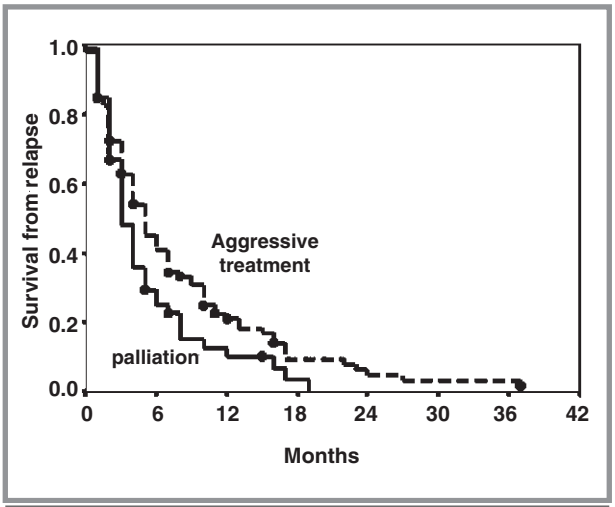

Figure 2. Overall survival from a series of 164 relapsed AML patients aged over 60 years observed at our institution according to treatment at relapse. The difference is statistically significant (p = 0.03) in favour of aggressive treatment vs palliation; however, the actual median survival (5 months vs 3 months) clearly indicates the absence of a clinically relevant advantage

are less impressive in multivariate analysis because of the pivotal prognostic role of the duration of first CR (CR1) i.e. longer or shorter than 12 months (p = 0.0001). This is shown in Figure 1, derived from a series of 101 relapsed AML patients observed at our institution. It is worth noting that the duration of CR1 significantly affected both achievement and duration of second CR (CR2), both in univariate and multivariate analyses in all studies focusing on prognostic factors in relapsed AML.[7-10] In addition, the prognostic value of CR1 duration has been confirmed for each additional year of CR.[39] However, it should be remembered that the majority of patients with CR1 lasting for less than one year belong to the adverse or intermediate cytogenetic category, therefore the lack of statistical significance in multivariate analysis in some studies may exclusively depend on the consistent overlapping of the two variables. In a recent report including 152 patients with relapsed AML, the CR2 rate of patients receiving salvage chemotherapy was 88, 64 and 36% among patients with cytogenetics defined by the MRC as favorable, intermediate or adverse. Moreover, there was a clear stepwise improvement in overall survival (OS) and disease-free survival (DFS) from the adverse to the favorable cytogenetic risk category.[43]

Age represents an additional relevant prognostic factor in relapsed AML.[44] However, it should be remembered that advanced age is associated with adverse cytogenetics and CR1 of short duration, which in turn account for poorer clinical outcome.[5,6] While a number of studies have looked at prognostic factors in newly diagnosed elderly AML patients,[5,6,33] few data are available on the specific issue of relapse in older AML patients. In a retrospective multicenter study on 150 AML patients aged over 60 years in first relapse, we found that 66% of them had been given aggressive therapy aimed at achieving CR2, while 34% had been managed with palliative treatment; of note, selection had been made mainly according to performance status at relapse.[44] Focusing on the subgroup of 100 aggressively treated patients, the CR1 duration was the only parameter significantly related to the CR2 rate, DFS and OS, while cytogenetics had a border-line effect (p = 0.09 in the multivariate analysis). In particular, the median survival was 8 months for patients whose CR1 lasted for more than twelve months as opposed to 4 months for the opposite group (p = 0.002), while median CR2 duration was 11 months vs. 5 months (p = 0.001), respectively. Overall, by retrospectively analyzing a large series of 164 relapsed patients aged over 60 years seen at our institution, we found that the median survival in patients receiving aggressive therapy and in those managed with only support was 5 and 3 months, respectively, and that this difference, despite being statistically significant (p = 0.03), is of only marginal clinical significance (Figure 2). Moreover, the advantage of aggressive salvage chemotherapy was irrelevant when analysis of therapeutic results was limited to the subgroup of patients with CR1 lasting for less than 12 months (4 months vs. 3 months, p = 0.10). These findings clearly suggest that advanced age by itself represents a main adverse prognostic factor in relapsed AML, with substantially no possibility of achieving long-term survival or cure as shown in Figure 3, in which 101 patients observed at our institution were compared by age. Accordingly,

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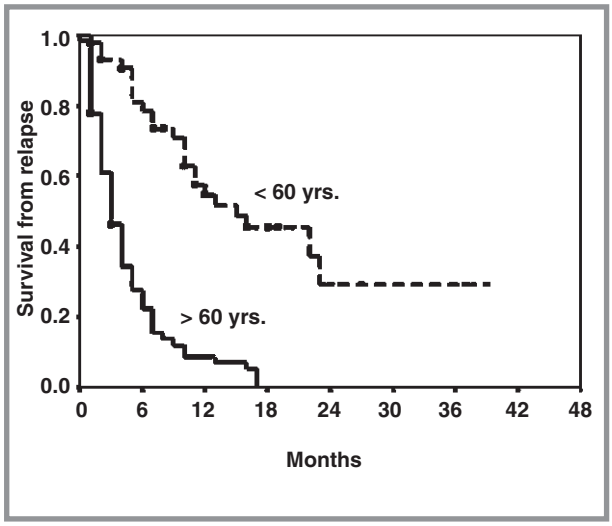

Figure 3. Overall survival from a series of 101 AML relapsed patients observed at our institution according to age, i.e.< or > 60 years (p = 0.001). The poor outcome of the older patient population suggests that these patients are ideal candidates for new therapeutic strategies.

relapsed elderly AML patients should be considered as ideal candidates for innovative therapeutic approaches. Finally, post-CR1 therapy represents a further factor influencing treatment results in relapsed AML, CR2 rate and duration being progressively worse after consolidation of CR1 with chemotherapy, autologous or allogeneic stem cell transplantation.[7-10]

The main prognostic factors for patients with relapsed AML are summarized in Table 1.

Therapeutic options for relapsed AML

Different therapeutic options (Table 2) are currently available for patients with relapsed AML, including conventional salvage chemotherapy, stem cell transplantation and new investigational agents.

Salvage chemotherapy

Therapeutic results for relapsed AML derive from retrospective studies, phase II single-agent studies, phase II combination-agent studies and a few phase III randomized trials.[8] Overall, CR2 rates range from 20 to 80%, with a median duration of CR2 ≤ 14 months and on overall median survival of ≤ 12 months. The probability of 3-year survival ranges from 8 to 30%. Best results in terms of CR2 rate and duration have been reported after combination therapies in patients with CR1 lasting for more than 12 months with favorable or intermediate cytogenetics (8-10). High or intermediate dose cytarabine (ARA-C)-based regimens still represent the most frequently adopted therapy.[45-49] In the last years, the combination of fludarabine with intermediate dose ARA-C and granulocyte colony-stimulating factor (G-CSF) (the FLAG regimen) with or without anthracyclines has been widely investigated as salvage treatment of AML in young adult or pediatric patients, usually resulting in CR2 rates higher than 50%.[50-56] The rational of these regimen relies on the synergistic action of fludarabine with ARA-C in increasing the intracellular level of the active metabolite ARA-CTP.[57] The role of G-CSF was either to induce leukemic cells to prolifer-

Table 1. Main adverse prognostic factors.

Age (> or < than 60 years)
Cytogenetics at diagnosis (MRC or SWOG criteria)
CR1 duration (more or less than 12 months)
Previous treatment (CHT, ASCT, alloSCT

Table 2. Therapeutic options for relapsed AML patients.

Salvage chemotherapy (generally high/intermediate dose ARA-C based)
Local therapy followed by consolidation (extrahematologic relapse)
Upfront alloSCT (relapse with low marrow infiltration)
Investigational agents
Combination of conventional chemotherapy + investigational agents
No therapy

Table 3. New agents with proven activity in relapsed AML.

Monoclonal antibodies (gemtuzumab ozogamycin)
Nucleoside analogs (clofarabine, troxacytabine)
FLT3 inhibitors (CEP-701, PKC412, SU5416, SU5614, SU11248)
Farnesyltransferase inhibitors (R115777-ZARNESTRA)
Inducers of apoptosis (G3139-Genasense)
Modulatore of MDR (cyclosporin A, PSC833)
Hypomethylating agents (decitabine)
Histone deacetylase (phenylbutyrate)

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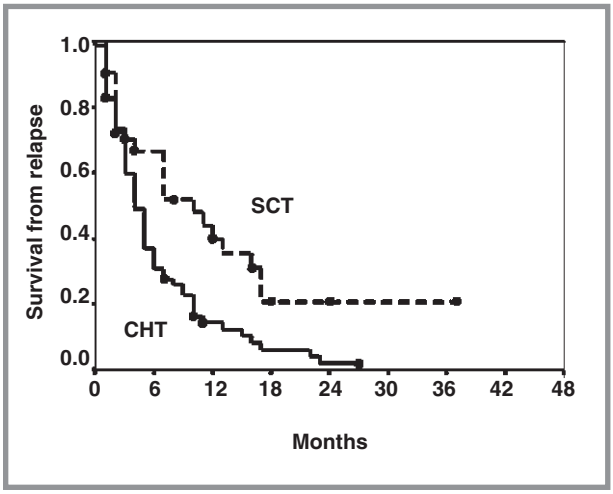

Figure 4. Therapeutic advantage of stem cell transplantation (auto or allo) vs consolidation chemotherapy in relapsed AML patients observed at our institution (p = 0.004).

ate rendering them more sensitive to ARA-C or to shorten the period of neutropenia induced by chemotherapy.[58] However, data from the MD Anderson group failed to demonstrate a significant increase in CR2 rate or survival when patients given FLAG were compared to those receiving only fludarabine and cytarabine.[59] Furthermore, it is still unclear whether the addition of idarubicin or mitoxantrone to FLAG results in a significant therapeutic advantage. In our experience, FLAG was able to induce CR rates over 50% in patients relapsing after either chemotherapy or autologous stem cell transplantation (ASCT),[51,56] as expected, results were poor in patients with unfavorable cytogenetics. Recently, we have developed a modified FLAG regimen based on sequential continuous infusion of fludarabine and ARA-C with encouraging results in terms of CR2 rate and toxicity.[60] Accordingly, a randomized trial comparing FLAG given as a continuous infusion vs. conventional FLAG has been planned.

In conclusion, at the moment there is not a gold standard salvage chemotherapy and the therapeutic choice mostly depends on personal experience concerning efficacy and toxicity. Notwithstanding this, whatever the salvage therapy, best results are achievable when stem cell transplantation (SCT) is feasible after salvage therapy.[7-10] On this basis, characteristics of an ideal salvage regimen should include either antileukemic efficacy or acceptable extra-hematologic toxicity. As a matter of fact, in clinical practice it is not rare that toxicity from previous therapy may preclude the feasibility of stem cell transplantation.

Stem cell transplantation

Once patients have relapsed after initial chemotherapy, further non-myeloablative therapy results in a poor outcome in the majority of patients. Results from our own experience (Figure 4) clearly demonstrate that stem cell transplantation (autologous or allogeneic) results in a significant therapeutic advantage over that offered by consolidation chemotherapy (p = 0.004).

Allogeneic transplantation represents the most effective option for patients in CR2 provided that suitable donors can be found. With matched sibling donors, long-term DFS of 30–40% can be expected although with an associated transplant-related mortality (TRM) of at least 30%.[61] In addition, for young adult patients with adverse prognostic factors (adverse cytogenetics, CR1 <12 months), lacking a compatible sibling, it seems reasonable to search for a matched unrelated donor or consider haploidentical transplantation[62,63] Results in terms of long-term disease free survival would balance the expected higher morbidity and mortality related to the procedure. Older patients or those with significant comorbidity coud take advantage from reduced intensity allogeneic stem cell transplantation (allo-SCT) with conditioning regimens specifically designed for AML, aimed at allowing full chimerism or inducing effective antileukemic activity.[64-66] Upfront alloSCT could be specifically recommended for patients with *early relapse*, where *early* indicates low marrow blast percentage (less than 10-20%) rather than time from CR1 achievement. Finally, donor lymphocyte infusion (DLI) can be considered for patients who relapse after allo-SCT. In patients with a small tumor burden DLI can result in a 15-20% CR rate, concomitantly with restoration of donor hematopoiesis.[67,68]

The potential utility of autologous stem cell transplantation (ASCT) has also been investigated in AML in situations other than CR.[69,70] While there is no rational indication for ASCT in overt relapse, different studies have reported promising results in CR2 patients. The Rome group initially reported a projected DFS of 52% at 63 months in a small series of 20 patients autografted after conditioning with the BAVC regimen.[71] More recently, an update from 60 patients with a longer follow-up reported a DFS of 42% at 10 years.[72] Other investigators have achieved DFS of 25–40%. Once again, the most important prognostic factors for patients undergoing ASCT in CR2 appear to be the duration of first remission and cytogenetics at diagnosis.[73-75]

New agents for the treatment of relapsed AML

Given the current poor results of conventional chemotherapy in relapsed AML, the search for innovative approaches based on mechanisms of actions targeted at specific pathways involved in leukemogenesis has rapidly progressed. The striking efficacy of

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oral retinoids in a difficult disease such as APL as well as the success of imatinib in chronic myeloid leukemia and some related disorders has provided substantial hope that therapy may be more effective and less toxic when well defined targets are identified[76-78] Table 3 lists the new agents for which clinical data are now available.

Monoclonal antibodies. One of the more fascinating approaches to the treatment of malignant diseases within the last decade has been the development of monoclonal antibody (MoAb) therapy. On a theoretical basis, by targeting antigens unique or preferentially expressed by malignant cells, these treatments would allow malignant clones to be eradicated while sparing normal tissue.[79]

CD33 is an antigen expressed on > 90% of AML cells with an average density of 10,000 sites per cell, while the antigen expression on normal pluripotent hematopoietic stem cells as well as of non-hemopoietic tissues is much less prominent.[80] In addition, CD33 is universally expressed at a high density in APL. On this basis, CD33 has been considered as an ideal target for the development of MoAb to be used in the clinical setting. Promising results were obtained by using the naked antibody in APL in patients with molecular relapse, while results in AML other than APL were less encouraging, probably because of rapid internalization into target cells on binding antigen.[81,82] With the aim of achieving a more advantageous toxicity/efficacy profile, antibody has been conjugated with toxins or radioisotopes to induce their delivery into leukemic cells. Robust data are now available on gemtuzumab ozogamicin (GO), an immunoconjugate composed of a humanized anti-CD33 antibody linked to the potent antitumor antibiotic calicheamicin. By encompassing data from three phase studies including 157 AML patients in first relapse with a median age of 68 years (range 60-87), Larson *et al.* reported a CR2 rate of 24%. The dose was 9 mg/m^2 on days 1 and 15 and toxicity was substantially hematologic with a median time to neutrophil and platelet recovery of 37 and 38 days, respectively. Of interest, in this study the incidence of venous occlusive disease (VOD) was low (only 3 cases, two of which were fatal).[83] A considerably higher incidence of VOD has been observed in other series mainly in patients consolidated with stem cell transplantation within 4 months after the last administration of GO.[84,85] In patients who developed VOD, histologic examination showed a striking deposition of sinusoidal collagen, suggesting that GO targets CD33$^+$ cells residing in hepatic sinusoids as the mechanism inducing hepatic toxicity.[84] Accordingly, it has been recommended that stem cell transplantation should be delayed by 4 months for patients achieving CR following GO administration.[85] An alternative approach could be to combine GO with chemotherapy. While the feasibility and efficacy of such an approach has been recently assessed in newly diagnosed patients as a prelude to the ongoing MRC AML 15 trial,[86] in relapsed patients results are much less encouraging, mainly due to the very substantial hepatic toxicity.[87,88] Finally, significantly lower CR rates have been reported after GO in patients with an unfavorable karyotype, suggesting that the strategy of immunoconjugates is not sufficient *per se* to overcome mechanisms of resistance deriving from adverse cytogenetics.[83,84]

Inhibitors of farnesyl transferase (Ftase)

Ftase is an enzyme that modifies proteins for localization to cell membrane. Many proteins are farnesylated and some of these are important in cancer development. Given that transforming activity of selected oncoproteins depends on farnesylation, blocking farnesylation may have therapeutic potential.[89] Different pharmacologic inhibitors of Ftase have been developed;[90] among these, consistent data are now available for R115777 (Zarnestra). This agent was investigated in 252 poor risk AML patients, 117 with primary refractory disease and 135 with relapsed disease, in a multicenter phase II study. The dose of Zarnestra was 600 mg bid for 21 days every 4 weeks in an outpatient setting; 169 patients (67%) completed at least one course with an overall CR rate of 6%; blast reduction over 50% was achieved in an additional 18% of the patients. Of interest, the CR rate was similar in relapsed and refractory patients. The overall median survival was 3 months, while the median survival for responding patients was 12 months. Grade III-IV non-hematologic toxicity was mild, consisting of fatigue (6%) and hypokaliemia (6%).[91]

New nucleoside analogs

Clofarabine, a potent inhibitor of ribonucleotide reductase, is active in adult patients with first relapsed and primary refractory acute leukemia and high-risk myelodysplastic syndrome (MDS). When given in combination with ARA-C, the drug results in a substantial increase of intracellular ARA-CTP, complementing the antileukemic activity of ARA-C.[92] Preliminary studies demonstrated that the drug was active as a single agent in different myeloid malignancies.[92,93] More recently, clofarabine has been investigated in combination with ARA-C in a series of 32 patients with poor risk myeloid malignancies, 28 of whom had recurrent/refractory AML.[94] The overall CR rate (CR + CRp, i.e. CR without platelet recovery > 100×10^9/L) was 33%; once again, best results were observed in patients with CR1 lasting for more than 12 months (57% vs. 27%) as well as in those with a diploid karyotype (54% vs. 20%).

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Troxacytabine, another nucleoside analog developed from the antiviral drug lamivudine, also showed significant activity in myeloid leukemias. However, results are less encouraging than those of clofarabine, either as a single agent or in combination with ARA-C, the CR rate not exceeding 20%.[95-97]

FLT3 inhibitors

The majority of known mutations in tyrosine kinases in AML occurr in members of the class III receptor tyrosine kinase family, which includes KIT, PDGFR, FLT3, and FMS. Among these, KIT and FLT3, are the two most commonly mutated receptors.[98] Length mutations in the juxtamembrane domain and activating loop mutations result in constitutive activation of the FLT3 kinase and activation of growth-related signaling pathways.[98,99] Robust data indicate that FLT3 mutations, including internal tandem duplications or point mutations in the activation loop, are independently associated with a poor prognosis in AML, in particular due to an increased rate of relapse.[100] Accordingly, there has been interest in developing FLT3 inhibitors with potential activity in AML. In phase I-II clinical trials on small numbers of patients, different FLT3 inhibitors, such as CEP-701, CT53518, PKC412, SU5416, SU5614, and SU11248 have shown an ability to induce blast cell reduction.[98]

Inducers of apoptosis

The clinical relevance of spontaneous apoptosis has been demonstrated in myeloid as well as lymphoid malignancies. Dysregulation of apoptosis in AML prolongs the survival of leukemic cells, resulting in their expansion independently of cell division[101] and is associated with a poor prognosis, unrelated to cell immaturity. Of interest, data from Del Poeta *et al.*, have also shown that spontaneous apoptosis is independent of immaturity in AML.[102] Thus, down-regulation of anti-apoptotic proteins, such as bcl-2, may reduce the threshold for chemotherapy resistance and restore chemosensitivity to leukemia cell.[103] Genasense (G3139) is an 18-mer phosphorothioate and potent inhibitor of proteasome, which is able to induce apoptosis and overcome resistance in a number of cell lines as well as primary cells from patients with CLL.[104] Transient decreases in blood or bone marrow blasts, along with significant downregulation of bcl-2 mRNA levels, were reported following treatment of relapsed patients with genansense. The drug is now under evaluation in a phase III trial in combination with chemotherapy in newly diagnosed AML patients.[104]

Modulators of multiple drug resistance (MDR). The expression of P-glycoprotein (Pgp) has a major role in inducing resistance to chemotherapeutic agents active in AML, such as anthracyclines and etoposide.[105] In particular, overexpression of Pgp results in decreased intracellular accumulation of cytotoxic drugs and this is closely related to *in vivo* and *in vitro* resistance to these drugs.[106] Accordingly, there has been great interest in the search for drugs able to revert MDR. Previous studies showed that the combination of MEC (mitoxantrone, etoposide and ARA-C) plus PSC833 is feasible in relapsed AML with a 29% CR rate. Disappointingly, a recently published randomized study from the CALGB group, comparing MEC vs. MEC + PSC833, failed to show any difference between the two arms in terms of either survival or disease-free survival.[107] At present, we have no data demonstrating that there is any advantage to be had from combining PSC833 or other MDR reverters with chemotherapy in poor risk AML.

Hypomethylating agents

Aberrant DNA methylation and other epigenetic events are important in the progression of a number of human malignancies. In particular, leukemias are characterized by high degrees of epigenetic changes marked by promoter methylation. In addition, hypermethylation of promoters of genes, such as p15, have been associated with disease progression and worse outcome in patients with myeloid malignancies.[108] These observations have led to renewed interest in developing DNA hypomethylating agents with potential clinical utility.[109] Decitabine (5-aza-2'-deoxycitidine) is a pyrimidine analog with significant antileukemic activity. Once incorporated into DNA, the drug irreversibly inhibits DNA methyltransferases, enzymes that methylate newly synthesized DNA.[110] Different studies have demonstrated the efficacy of different dose levels of decitabine in patients with recurrent or refractory leukemia. However, the dose of 15 mg/m^2 for 10 days was recently reported as more advantageous in terms of response rate and toxicity in a phase I study including 44 patients with AML/MDS. The conclusion of the authors was that decitabine is an active drug in poor risk AML and low doses are as effective as or more effective than higher doses.[111]

Histone deacetylase

The acetylation of histones is involved in the regulation of gene expression through chromatin remodeling. Alterations in histone deacetylation have been shown to play a role in the pathogenetic mechanisms of leukemogenesis. Different chromosomal translocations in AML result in hybrid fusion proteins, which are involved in blocking the differentiation of the hematopoietic precursors. The abnormal fusion proteins bind to DNA and interact with transcriptional co-regulators, resulting in an increased local concentration of histone deacetylase (HDAC) complexes. HDAC inhibitors have been developed in order to tar-

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get the above mechanism. Their aim is to remove the block of hematopoietic differentiation.[112] Due to the success of HDAC inhibitors in preclinical models, clinical phase I and II trials are ongoing in AML with phenylbutyrate in combination with retinoic acid or azacytidine, desipepside, pyroxamide, valproic acid and others. Results are still preliminary and no definite information is available on the optimal dose, best application form, efficacy side effects. Notwithstanding this, histone deacetylation represents a stimulating field of clinical research in AML and it will be of great interest to investigate novel agents synergizing with HDAC inhibitors.[78,112]

Relapse in APL

CR rates over 90% are currently achievable in APL with the combination of ATRA plus chemotherapy. This results in long-term survival and cure in about 70% of newly diagnosed patients.[25] Nevertheless, treatment failure still occurs in approximately 30% of cases as a result of early hemorrhagic death or disease relapse.[25] As far as relapse is concerned, the majority of patients can be reinduced into CR by different approaches including ATRA, arsenic trioxide (ATO), chemotherapy or a combination of these.[113] The treatment of choice for relapsed APL should be ATO, which results in CR2 rates of over 80% as a single agent and is an ideal bridge to stem cell transplantation.[114] The most relevant toxicities include prolongation of the QT interval and the APL differentiation syndrome, a cardiorespiratory distress syndrome with multiple pulmonary infiltrates, reminiscent of RAS and similarly responsive to dexamethasone. In addition, preliminary studies in a small cohort of patients testing arsenic trioxide at lower doses suggest that the efficacy of this strategy is similar to that of the standard dose, but with less toxicity.[97] The use of GO, reported to be effective for treating molecular relapse, needs further investigation.[110]

Once CR2 has been achieved the optimal strategy to prevent further relapse is still controversial.[117] The benefits of allo-SCT rely on the high dose chemotherapy and/or radiotherapy of the conditioning regimen as well as on the graft-versus-leukemia effect (GVL). However, the morbidity and mortality from the procedure are still high, especially in elderly individuals; furthermore, the requirement of an HLA-identical donor represents a major obstacle to the performance of alloSCT. On the other hand, ASCT offers the possibility of delivering high dose chemotherapy and/or radiotherapy without the morbidity and mortality due to graft-versus-host disease (GVHD), even though the relapse rate after ASCT is expected to be higher, given the lack of GVL effect. Previous studies have suggested that ASCT is effective in inducing prolonged relapse-free survival in relapsed APL patients autografted in second molecular remission, independently from positivity of bone marrow graft for the PML/RARalpha fusion gene,[117-119] which is the molecular marker of APL. Of a small series of 6 patients autografted in second (n=5) or fourth (n=1) molecular remission with a molecularly negative apheresis product or bone marrow harvest, 5 out of 6 obtained long-term molecular remission, suggesting that ASCT performed with a molecularly negative graft in APL patients in second molecular remission offers a valid chance for achieving a cure.[119] Given the still considerable mortality from alloSCT, such an approach should also be considered in relapsed patients with an HLA compatible donor, namely in those with CR1 that has lasted for more than one year or in elderly individuals.

Conclusions

Relapse still represents the most relevant obstacle to achieving a cure in AML. In our daily work, we continuously experience just how frustrating relapses are for the patient and physician; in addition, salvage therapy followed or not by stem cell transplantation is poorly accepted, potentially associated with high morbidity and mortality and costly. Nonetheless, relapse offers the opportunity for the investigation of new therapeutic strategies, mainly in patients who are expected to gain negligible advantage from a conventional salvage chemotherapy. The wide differences in CR2 rates and durations in various phase II single-arm trials have clearly shown that biological and clinical characteristics of the patients as well as different treatment modalities account for discordant results and this can substantially hamper the selection of therapies to investigate in phase III trials. The development of well designed, randomized phase II trials would allow several therapies to be evaluated, based on multiple outcomes, providing new and exciting knowledge in this fascinating field of clinical hematology.[120]

FF conceived and drafted the paper. SP and GM collected data from personal experience and literature and critically revised the manuscript.

Data for this review were identified using PubMed and references from most relevant articles in English from 1990 to 2004. The personal experience of the authors was also taken into account.

The authors reported no potential conflicts of interest

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References

1. Schiffer CA. Hematopoietic growth factors and the future of therapeutic research on acute myeloid leukemia. New Engl J Med 2003;349:727-9.
2. Kohlmann A, Schoch C, Schnittger S, Dugas M, Hiddemann W, Kern W, et al. Molecular characterization of acute leukemias by use of microarray technology. Genes Chromosomes Cancer 2003; 37:396-405.
3. Ravandi F, Talpaz M, Estrov Z. Modulation of cellular signalling pathways: prospects for targeted therapy in haematological malignancies. Clin Cancer Res 2003; 9: 535–50.

4. Stirewalt DL, Meshinchi S, Radich JP. Molecular targets in acute myelogenous leukemia. Blood Rev 2003;17:15-23.
5. Pinto A, Zagonel V, Ferrara F. Acute myeloid leukaemia in the elderly: biology and therapeutic strategies. Crit Rev Oncol Hematol 2001;39:275–87.
6. Ferrara F, Mirto S, Zagonel V, Pinto A. Acute myeloid leukemia in the elderly: a critical review of therapeutic approaches and appraisal of results of therapy. Leuk Lymphoma 1998;29:375–82.
7. Estey EH. Treatment of relapsed and refractory acute myelogenous leukemia. Leukemia 2000;14:476-9.
8. Leopold LH, Willemze R. The treatment of acute myeloid leukemia in first relapse: a comprehensive review of the literature. Leuk Lymphoma 2002;43: 1715-27.
9. Stanisic S, Kalaycio M. Treatment of refractory and relapsed acute myelogenous leukemia. Expert Rev Anticancer Ther 2002;2:287-95.
10. Robak T, Wrzesien K. The search for optimal treatment in relapsed and refractory acute myeloid leukemia. Leuk Lymphoma 2002;43:281-91.
11. Newland A. Progress in the treatment of acute myeloid leukaemia in adults. Int J Hematol 2002; 76 Suppl 1:253-8.
12. Stone RM. Treatment of acute myeloid leukemia: state-of-the-art and future directions. Semin Hematol 2002; 39 Suppl 2:4-10.
13. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 2003;21: 4642-9.
14. Ginsberg JP, Orudjev E, Bunin N, Felix CA, Lange BJ. Isolated extramedullary relapse in acute myeloid leukemia: a retrospective analysis. Med Pediatr Oncol 2002; 38:387-90.
15. Lee KH, Lee JH, Choi SJ, Lee JH, Kim S, Seol M, et al. Bone marrow vs extramedullary relapse of acute leukemia after allogeneic hematopoietic cell transplantation: risk factors and clinical course. Bone Marrow Transplant 2003; 32:835-42.
16. Ferrara F, Del Vecchio L. Acute myeloid leukemia with t(8;21)/AML1/ETO: a distinct biological and clinical entity. Haematologica 2002;87:306-19.
17. Wiernik PH, De Bellis R, Muxi P, Dutcher JP. Extramedullary acute promyelocytic leukemia. Cancer 1996;78:2510-4.
18. Evans GD, Grimwade DJ. Extramedullary disease in acute promyelocytic leukemia. Leuk Lymphoma 1999;33:219-29.
19. Specchia G, Lo Coco F, Vignetti M, Avvisati G, Fazi P, Albano F, et al. Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with ATRA. A report by the GIMEMA group. J Clin Oncol 2001; 19: 4023–8.
20. Breccia M, Carmosino I, Diverio D, De Santis S, De Propris MS, Romano A, et al. Early detection of meningeal localization in acute promyelocytic leukaemia patients with high presenting leucocyte count. Br J Haematol 2003;120:266-70.
21. Grimwade D, Lo Coco F. Acute promyelocytic leukemia: a model for the role of molecular diagnosis and residual disease monitoring in directing treatment approach in acute myeloid leukemia. Leukemia 2002;16:1959-73.
22. Grinwade D. The significance of minimal residual disease in patients with t(15;17). Best Pract Res Clin Haematol 2002; 15: 137-58.
23. Mandelli F, Diverio D, Avvisati G, Luciano A, Barbui T, Bernasconi C, et al. Molecular remission in PML/RARα-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Blood 1997; 90: 1014–21.
24. Lo Coco F, Diverio D, Falini B, Biondi A, Nervi C, Pelicci PG. Genetic diagnosis and molecular monitoring in the management of acute promyelocytic leukemia. Blood 1999;94:12–6.
25. Tallman M, Nabhan C. Management of acute promyelocytic leukemia. Curr Oncol Rep 2002;4:381-9.
26. Marcucci G, Caligiuri MA, Bloomfield CD. Core binding factor (CBF) acute myeloid leukemia: is molecular monitoring by RT-PCR useful clinically? Eur J Haematol 2003;71:143-54.
27. Buonamici S, Ottavini E, Testoni N, Montefusco V, Visani G, Bonifazi F, et al. Real-time quantitation of minimal residual disease in inv(16)-positive acute myeloid leukemia may indicate risk for clinical relapse and may identify patients in a curable state. Blood 2002;99:443-9.
28. Schnittger S, Weisser M, Schoch C, Hiddemann W, Haferlach T, Kern W. New score predicting for prognosis in PML-RARA+, AML1-ET0+, or CBFBMYH11+ acute myeloid leukemia based on quantification of fusion transcripts. Blood 2003;102:2746-55.
29. Krauter J, Gorlich K, Ottmann O, Lubbert M, Dohner H, Heit W, et al. Prognostic value of minimal residual disease quantification by real-time reverse transcriptase polymerase chain reaction in patients with core binding factor leukemias. J Clin Oncol 2003;23:4413-22.
30. Morschhauser F, Cayuela JM, Martini S, Baruchel A, Rousselot P, Socie G, et al. Evaluation of minimal residual disease using reverse-transcription polymerase chain reaction in t(8;21) acute myeloid leukemia: a multicenter study of 51 patients. J Clin Oncol 2000;18:788-94.
31. Cilloni D, Gottardi E, De Micheli D, Serra A, Volpe G, Messa F, et al. Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. Leukemia 2002;16:2115-21.
32. Garg M, Moore H, Tobal K, Liu Yin JA. Prognostic significance of quantitative analysis of WT1 gene transcripts by competitive reverse transcription polymerase chain reaction in acute leukaemia. Br J Haematol 2003;123:49-59.
33. Ogawa H, Tamaki H, Ikegame K, Soma T, Kawakami M, Tsuboi A et al. The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. Blood 2003;101:1698-704.
34. Löwenberg B. Prognostic factors in acute myeloid leukemia. Best Pract Res Clin Haematol 2001;14:65-75.
35. Kern W, Haferlach T, Schoch C, Loffler H, Gassmann W, Heinecke A, et al. Early blast clearance by remission induction therapy is a major independent prognostic factor for both achievement of complete remission and long-term outcome in acute myeloid leukemia: data from the German AML Cooperative Group (AML-CG) 1992 Trial. Blood 2003;101:64-70.
36. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 1998;92:2322-33.
37. Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. Blood 2000;96:4075-83.
38. Kern W, Schoch C, Haferlach T, Unterhalt M, Wormann B, Buchner T, et al. Multivariate analysis of prognostic factors in patients with refractory and relapsed acute myeloid leukemia undergoing sequential high-dose cytosine arabinoside and mitoxantrone (S-HAM) salvage therapy: relevance of cytogenetic abnormalities. Leukemia 2000; 14:226-31.
39. Kern W, Schoch C, Schnittger S, Ludwig WD, Hiddemann W, Schoch C. Karyotype instability between diagnosis and relapse in 117 patients with acute myeloid leukemia: implications for resistance against therapy. Leukemia 2002; 14: 2084-91.
40. Hiddemann W, Martin WR, Sauerland CM, Heinecke A, Buchner T. Definition of refractoriness against conventional chemotherapy in acute myeloid leukemia: a proposal based on the results of retreatment by thioguanine, cytosine arabinoside, and daunorubicin (TAD 9) in 150 patients with relapse after standardized first line therapy. Leukemia 1990;4:184–8.
41. Davis CL, Rohatiner AZ, Lim J, Whelan JS, Oza AM, Amess J, et al. The management of recurrent acute myelogenous leukaemia at a single centre over a fifteen-year period. Br J Haematol 1993; 83: 404–11.
42. Thalhammer F, Geissler K, Jager U, Kyrle PA, Pabinger I, Mitterbauer M, et al. Duration of second complete remission in patients with acute myeloid leukemia treated with chemotherapy: a retrospective single-center study. Ann Hematol 1996;72:216–22.
43. Weltermann A, Fonatsch C, OA Haas, Greinix HT, Kahls P, Mitterbauer G, et al. Impact of cytogenetics on the prognosis of adults with de novo AML in first relapse. Leukemia 2004;12:293-302.
44. Ferrara F, Morabito F, Latagliata R, Martino B, Annunziata M, Oliva E, et al. Aggressive salvage treatment is not appropriate for the majority of elderly patients with acute myeloid leukaemia relapsing after first complete remission Haematologica 2001;86:814-20.

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45. Vignetti M, Orsini E, Petti MC, Moleti ML, Andrizzi C, Pinto RM, et al. Probability of long-term disease-free survival for acute myeloid leukemia patients after first relapse: a single-centre experience. Ann Oncol 1996;7:933–8.
46. Carella AM, Carlier P, Pungolino E, Resegotti L, Liso V, Stasi R, et al. Idarubicin in combination with intermediate-dose cytarabine and VP-16 in the treatment of refractory or rapidly relapsed patients with acute myeloid leukemia. Leukemia 1993;7:196–9.
47. De Witte T, Suciu S, Selleslag D, Labar B, Roozendaal K, Zittoun R, et al. Salvage treatment for primary resistant acute myelogenous leukemia consisting of intermediate-dose cytosine arabinoside and interspaced continuous infusions of idarubicin: a phase-II study (no. 06901) of the EORTC Leukemia Cooperative Group. Ann Hematol 1996;72:119–24.
48. Karanes C, Kopecky KJ, Head DR, Grever MR, Hymed HE, Kraut EH, et al. A phase III comparison of high dose ARAC (HIDAC) versus HIDAC plus mitoxantrone in the treatment of first relapsed or refractory acute myeloid leukemia Southwest Oncology Group Study. Leuk Res 1999;23:787–94.
49. Martino R, Guardia R, Altes A, Sureda A, Brunet S, Sierra J. Time sequential chemotherapy for primary refractory or relapsed adult acute myeloid leukemia: results of the phase II GEMIA protocol. Haematologica 1999;84:226–30.
50. Virchis A, Koh M, Rankin P, Mehta A, Potter M, Hoffbrand AV, et al. Fludarabine, cytosine arabinoside, granulocyte-colony stimulating factor with or without idarubicin in the treatment of high risk acute leukaemia or myelodysplastic syndromes, Br J Haematol 2004;124:26-32.
51. Montillo M, Mirto S, Petti MC, Latagliata R, Magrin S, Pinto A, et al. Fludarabine, cytarabine, and G-CSF (FLAG) for the treatment of poor risk acute myeloid leukemia. Am J Hematol 1998;58:105–9.
52. Ferrara F, Leoni F, Pinto A, Mirto S, Morra E, Zagonel V, et al. Fludarabine, cytarabine, and granulocyte-colony stimulating factor for the treatment of high risk myelodysplastic syndromes. Cancer 1999;86:2006–13.
53. Clavio M, Carrara P, Miglino M, Pierri I, Canepa L, Balleari E, et al. High efficacy of fludarabine-containing therapy (FLAG-FLANG) in poor risk acute myeloid leukemia. Haematologica 1996; 81: 513–20.
54. Jackson G, Taylor P, Smith GM, Marcus R, Smith A, Chu P, et al. A multicentre, open, non-comparative phase II study of a combination of fludarabine phosphate, cytarabine and granulocyte colony-stimulating factor in relapsed and refractory acute myeloid leukaemia and de novo refractory anaemia with excess blasts in transformation. Br J Haematol 2001;112:127–37.
55. Neufang A, Franklin J, Tesch H, Diehl V, Dias WP. Phase-II trial of idarubicin, fludarabine, cytosine arabinoside, and filgrastim (Ida-FLAG) for treatment of refractory, relapsed, and secondary AML. Ann Hematol 1999;78:418–25.
56. Ferrara F, Melillo L, Montillo M, Leoni F, Pinto A, Mele G, et al. Fludarabine, cytarabine, and G-CSF (FLAG) for the treatment of acute myeloid leukemia relapsing after autologous stem cell transplantation. Ann Hematol 1999; 78: 380-4.
57. Gandhi V, Estey E, Keating MJ, Plunkett W. Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 1993;11:116–24.
58. Tafuri A, Andreeff M. Kinetic rationale for cytokine induced recruitment of myeloblastic leukemia followed by cycle specific chemotherapy in vitro. Leukemia 2000;4:826–34.
59. Estey E, Thall P, Andreeff M, Beran M, Kantarjian H, O'Brien S, et al. Use of granulocyte colony-stimulating factor before, during, and after fludarabine plus cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colonystimulating factor. J Clin Oncol 2004;12:671–8.
60. Camera A, Palmieri S, Cantore N, Mele G, Rinaldi CR, Villa MR, et al. Fludarabine and cytarabine as continuous sequential infusion plus liposomal daunorubicin (Daunoxome) as salvage therapy for refractory or relapsed patients with acute myeloid leukemia. Haematologica 2003; 88 Suppl 15:84-5.
61. Mengarelli A, Iori A, Guglielmi C, Romano A, Cerretti R, Torromeo C, et al. Standard versus alternative myeloablative conditioning regimens in allogeneic hematopoietic stem cell transplantation for high-risk acute leukemia. Haematologica 2002;87:52-8.
62. Chalandon Y, Barnett MJ, Horsman DE, Conneally EA, Nantel SH, Nevill TJ, et al. Influence of cytogenetic abnormalities on outcome after allogeneic bone marrow transplantation for acute myeloid leukemia in first complete remission. Biol Blood Marrow Transplant 2002; 8:435-43.
63. Aversa F, Velardi A, Tabilio A, Reisner Y, Martelli MF. Haploidentical stem cell transplantation in leukemia. Blood Rev 2001;15:111-9.
64. Massenkeil G, Nagy M, Lawang M, Rosen O, Genvresse I, Geserick G, et al. Reduced intensity conditioning and prophylactic DLI can cure patients with high-risk acute leukaemias if complete donor chimerism can be achieved. Bone Marrow Transplant 2003;31:339-45.
65. Wong R, Giralt SA, Martin T, Couriel DR, Anagnostopoulos A, Hosing C, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood 2003;102:3052-9.
66. Taussig DC, Davies AJ, Cavenagh JD, Oakervee H, Syndercombe-Court D, Kelsey S, et al. Durable remissions of myelodysplastic syndrome and acute myeloid leukemia after reduced-intensity allografting. J Clin Oncol 2003;15:3060-5.
67. Imoto S, Murayama T, Gomyo H, Mizuno I, Sugimoto T, Nakagawa T, et al. Long-term molecular remission induced by donor lymphocyte infusions for recurrent acute myeloblastic leukemia after allogeneic bone marrow transplantation. Bone Marrow Transplant 2000;26:809-10.
68. Wasch R, Bertz H, Kunzmann R, Finke J. Incidence of mixed chimaerism and clinical outcome in 101 patients after myeloablative conditioning regimens and allogeneic stem cell transplantation. Br J Haematol 2000;109:743-50.
69. Linker CA. Autologous stem cell transplantation for acute myeloid leukemia. Bone Marrow Transplant 2003;31:731-8.
70. Gorin NC. Autologous stem cell transplantation for adult acute leukemia Curr Opin Oncol 2002;14:152-9.
71. Meloni G, De Fabritiis P, Petti MC, Mandelli F. BAVC regimen and autologous bone marrow transplantation in patients with acute myelogenous leukemia in second remission. Blood 1990;75:2282-5.
72. Meloni G, Vignetti M, Avvisati G, Capria S, Micozzi A, Giona F, et al. BAVC regimen and autograft for acute myelogenous leukemia in second complete remission. Bone Marrow Transplant 1996; 18:693-8.
73. Smith BD, Jones RJ, Lee SM, Piantadosi S, Vala MS, Fuller D. Autologous bone marrow transplantation with 4-hydroperoxycyclophosphamide purging for acute myeloid leukaemia beyond first remission: a 10-year experience. Br J Haematol 2002;117:907-13.
74. Lioure B, Witz F, Deconninck E. Autologous stem cell transplantation (ASCT) represents the best consolidation therapy for acute myelogenous leukemia (AML) in second complete remission (CR): a retrospective study of the GOELAMS group. Blood 1997; 90 Suppl 1: 384a[abstract].
75. Linker C, George S, Hurd D, Ries C, Negrin R, Blume K, et al. Autologous stem cell transplantation for acute myeloid leukemia in second remission-CALGB 9620. Blood 2001;98 Suppl 1: 689a[abstract].
76. Giles FJ. New drugs in acute myeloid leukemia. Curr Oncol Rep 2002;4:369-74.
77. Stone RM. New therapies in leukemia: renewed hope. J Hematother Stem Cell Res 2002;11:583-7.
78. Ravandi F, Kantarjian H, Giles F, Cortes J. New agents in acute myeloid leukemia and other myeloid disorders. Cancer 2004;100:441–54.
79. Tomblyn MR, Tallman MS. New developments in antibody therapy for acute myeloid leukemia. Semin Oncol 2003; 30:502-8.
80. Scheinberg DA, Tanimoto M, McKenzie S, Strife A, Old LJ, Clarkson BD. Monoclonal antibody M195: a diagnostic marker for acute myelogenous leukemia. Leukemia 1989;3:440–5.
81. Jurcic JG, DeBlasio T, Dumont L, Yao TJ, Scheinberg DA. Molecular remission induction with retinoic acid and anti-mCD33 monoclonal antibody HuM195 in acute promyelocytic leukemia. Clin Cancer Res 2000;6:372–80.
82. Feldman E, Kalaycio M, Weiner G, Frankel S, Schulman P, Schwartzberg L, et al. Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. Leukemia 2003;17:314–8.
83. Larson RA, Sievers EL, Stadtmauer EA, Lowenberg B, Estey EH, et al. Use of gemtuzumab ozogamicin (Mylotarg) in 157 patients 60 years of age with acute

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myeloid leukemia in first relapse. Blood 2003;102:176a.
84. Rajvanshi P, Shulman HM, Sievers EL, McDonald GB. Hepatic sinusoidal obstruction after gemtuzumab ozogamicin (Mylotarg) therapy. Blood 2002; 99: 2310-4.
85. Erba HP, Stadtmauer EA, Larson RA et al. Risk Assessment for hepatic veno-occlusive disease in patients treated with gemtuzumab ozogamicin (Mylotarg) with or without hematopoietic stem cell transplantation. Blood 2003; 102:871a[abstract].
86. Kell WJ, Burnett AK, Chopra R, Yin JA, Clark RE, Rohatiner A, et al. A feasibility study of simultanenous administration of gemtuzumab ozogamicin with intensive chemotherapy in induction and consolidation in younger patients with acute myeloid leukemia. Blood 2003;102:4277-83.
87. Tsimberidou A, Cortes J, Thomas D, Garcia-Manero G, Verstovsek S, Faderl S, et al Gemtuzumab ozogamicin, fludarabine, cytarabine and cyclosporine combination regimen in patients with CD33+ primary resistant or relapsed acute myeloid leukemia. Leuk Res 2003;27:893-7.
88. Alvarado Y, Tsimberidou A, Kantarjian H, Cortes J, Garcia-Manero G, Faderl S, et al. Pilot study of Mylotarg, idarubicin and cytarabine combination regimen in patients with primary resistant or relapsed acute myeloid leukemia. Cancer Chemother Pharmacol 2003;51:87-90.
89. Karp JE. Farnesyl protein transferase inhibitors as targeted therapies for hematologic malignancies. Semin Hematol 2001;38 Suppl 7:16-23.
90. Lancet JE, Karp JE. Farnesyl transferase inhibitors in myeloid malignancies. Blood Rev 2003;17:123-9.
91. Harousseau J, Reiffers J, Lowenberg B, Thomas X, Huguet F, Fenaux P, et al. Zarnestra (R115777) in patients with relapsed and refractory acute myelogenous leukemia (AML): results of a multicenter phase 2 Study. Blood 2003; 176a[abstract].
92. Kantarjian HM, Gandhi V, Kozuch P, Faderl S, Giles F, Cortes J, et al. Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers. J Clin Oncol 2003; 21: 1167–73.
93. Kantarjian HM, Gandhi V, Cortes J, Verstovsek S, Du M, Garcia-Manero G, et al. Phase II clinical and pharmacology study of clofarabine in patients with refractory or relapsed acute leukemia. Blood 2003;102:2379–86.
94. Faderl S, Gandhi V, Garcia-Manero G, Thomas DA, Cortes JE, O Brien S, et al. Clofarabine is active in combination with cytarabine (ara-C) in adult patients (pts) in first relapsed and primary refractory acute leukemia and high-risk myelodysplastic syndrome (MDS). Blood 2003; 102:615a[abstract].
95. Giles FJ, Cortes JE, Baker SD, Thomas DA, O'Brien S, Smith TL, et al. Troxacitabine, a novel dioxolane nucleoside analog, has activity in patients with advanced leukemia. J Clin Oncol 2001;19:762–71.
96. Giles FJ, Garcia-Manero G, Cortes JE, Baker SD, Miller CB, O'Brien SM, et al. Phase II study of troxacitabine, a novel dioxolane nucleoside analog, in patients with refractory leukemia. J Clin Oncol 2002;20:656–64.
97. Giles FJ, Faderl S, Thomas DA, Cortes JE, Garcia-Manero G, Douer D, et al. Randomized phase I/II study of troxacitabine combined with cytarabine, idarubicin, or topotecan in patients with refractory myeloid leukemias. J Clin Oncol 2003; 21: 1050–6.
98. Löwenberg B, Griffin JD, Tallman MS. Acute myeloid leukemia and acute promyelocytic leukemia. Hematology (Am Soc Hematol Educ Program) 2003; 82-101.
99. Stirewalt DL, Radich JP. The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer 2003;3:650-65.
100. Sheikhha MH, Awan A, Tobal K, Liu Yin JA. Prognostic significance of FLT3 ITD and D835 mutations in AML patients. Hematol J 2003;4:41-6.
101. Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol 1999;17:2941–53.
102. Del Poeta G, Del Principe MI, Buccisano F, Venditti A, Maurillo L, Tamburini A, et al. Clinical significance of spontaneous apoptosis is independent from immaturity in acute myeloid leukemia (AML). Blood 2003;102:100a[abstract].
103. Cotter FE. Antisense therapy of hematologic malignancies. Semin Hematol 1999; 36:9–14.
104. Marcucci G, Byrd JC, Dai G, Klisovic MI, Kourlas PJ, Young DC, et al. Phase 1 and pharmacodynamic studies of G3139, a Bcl-2 antisense oligonucleotide, in combination with chemotherapy in refractory or relapsed acute leukemia. Blood 2003; 101:425–32.
105. Sonneveld P. Multidrug resistance in haematological malignancies. J Intern Med 2000;247:521–34.
106. Nooter K, Sonneveld P, Oostrum R, Herweijer H, HagenbeekT, Valerio D. Overexpression of the mdr1 gene in blast cells from patients with acute myelocytic leukemia is associated with decreased anthracycline accumulation that can be restored by cyclosporin-A. Int J Cancer 1990;45:263–8.
107. Greenberg PL, Lee SJ, Advani R, Tallman MS, Sikic BI, Letendre L, et al. Mitoxantrone, etoposide, and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995). J Clin Oncol 2004;22:1078-86.
108. Issa JPJ, Baylin SB, Herman JG. DNA methylation changes in hematologic malignancies: biologic and clinical implications. Leukemia 1997;11 Suppl 1:S7-S11.
109. Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. Ann Intern Med 2001; 134: 573-86.
110. Christman JK. E and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. Oncogene 2002; 21:5483-95.
111. Issa JPJ, Garcia-Manero G, Giles FJ, Mannari R, Thomas D, Faderl S, et al. Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. Blood 2004; 103:1635-40.
112. Pandolfi PP, Moe-Behrenz GHG. Targeting aberrant transcriptional repression in acute myeloid leukemia. Rev Clin Exp Hematol 2003;7:139-59.
113. Estey EH. Treatment options for relapsed acute promyelocytic leukaemia. Best Pract Res Clin Haematol 2003;16:521-34.
114. Leoni F, Gianfaldoni G, Annunziata M, Fanci R, Ciolli S, Nozzoli C, et al. Arsenic trioxide therapy for relapsed acute promyelocytic leukemia: a bridge to transplantation. Haematologica 2002; 87:485-9.
115. Shen Y, Shen ZX, Yan H, Chen J, Zeng XY, Li JM, et al. Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. Leukemia. 2001;15:735-41.
116. Petti MC, Pinazzi MB, Diverio D, Romano A, Petrucci MT, De Santis S, et al. Prolonged molecular remission in advanced acute promyelocytic leukaemia after treatment with gemtuzumab ozogamicin (Mylotarg CMA-676). Br J Haematol 2001; 115:63–5.
117. Sanz MA, de la Rubia J, Bonanad S, Barragan E, Sempere A, Martin G, et al. Prolonged molecular remission after PML/RARα-positive autologus peripheral blood stem cell transplantation in acute promyelocytic leukemia: is relevant pretransplant minimal residual disease in the graft? Leukemia 1998; 12: 992-5.
118. Ruiz-Arguelles G, Garces-Eisele J, Ruiz-Arguelles A. Continued complete remission after a PML/RAR-α+ autograft in acute promyelocytic leukemia. Eur J Haematol 1996;55:186-7.
119. Ferrara F, Palmieri S, Annunziata M, Pocali B, Viola A, Pane F. Prolonged molecular remission after autologous stem cell transplantation in relapsed acute promyelocytic leukemia: report on six patients autografted in molecular remission by molecularly negative graft. Haematologica 2004;89:621-2.
120. Estey EH, Thall PF. New designs for phase 2 clinical trials. Blood 2003;102:442–8.

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