Best Practice & Research Clinical Haematology
Vol. 14, No. 1, pp. 95–118, 2001
doi:10.1053/beha.2000.0118, available online at http://www.idealibrary.com on IDEAL®

BEST PRACTICE & RESEARCH

6

Treatment of acute myeloid leukaemia in younger patients

Alan K. Burnett MD, FRCP (Edin, Lon, Glas), FRCPath, FMSci

Professor and Head of Department
Department of Haematology, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN, UK

The survival of AML in younger patients has improved in the last 20 years, as a consequence of a more intensive approach to treatment. Seventy-five to eighty percent of patients will enter complete remission, so the main challenge is to prevent relapse. Several trials have assessed the value of allogeneic or autologous transplantation. When these trials have been assessed by careful statistical methods, the advantage of transplant overall is difficult to detect. Intensive consolidation can deliver a similar survival, of which high-dose Ara-C has been widely adopted, but other intensive schedules appear equivalent. It is not known how many treatment courses are required. Patients are at differing risks of relapse which may influence the choice of treatment. In trials where a risk profile is available, and where a donor versus no-donor analysis is performed, there appears to be little robust evidence to support transplant in good or poor risk disease, although the experience in the latter groups is not unanimous. Standard risk patients may be the subgroup who deliver survival benefit, but since chemotherapy continues to improve, there remains some uncertainty. It is possible that technical improvements in transplantation, such as peripheral blood as a source of stem cells, may remove this uncertainty.

Key words: consolidation; risk group; allograft; autograft.

Several recent clinical trials have segregated patients into younger (<60 years) or older (>60 years) groups largely because most of the therapeutic options are tolerable in patients <60 years. Remission rates, disease-free and overall survival have improved in these patients in the last 15 years such that 70–80% of patients will enter complete remission and 35–40% of patients will survive.[1–3] As treatment has become successful prognostic factors have emerged that can predict the initial and long-term response (see Chapter 4). The pattern of relapse has changed as treatment has intensified, with most events occurring within the first 3 years. The definition of remission depends on marrow morphology, lack of extramedually disease and regeneration of peripheral blood counts.[4] There has been a trend toward initiating the second course of chemotherapy before full peripheral regeneration has been achieved. Considerable evidence is now accumulating from molecular techniques that residual disease is present when the conventional criteria are reached.[5–7] Clonal rather than polyclonal haemopoiesis may be present, but the clinical significance of this phenomenon is not clear.[8] The United Kingdom Medical Research Council (UK MRC) Trial Group have not required peripheral blood recovery as a criterion of remission, which perhaps explains the slightly better remission rates reported by that group; however they find

1521–6926/01/010095+24 $35.00/00

little difference in subsequent outcome as long as the marrow blasts are less than 15%.[1,9] Patients who fail to achieve this are not given a further course of the same treatment but changed to an alternative regimen.

INDUCTION CHEMOTHERAPY

The combination of daunorubicin and cytosine arabinoside (Ara-C) has been the backbone of induction treatment for 30 years.[10,11] Ara-C is given by 7 day infusion or 12 hourly 10 day schedules. Randomized studies have evaluated mitoxantrone or idarubicin instead of daunorubicin in otherwise conventional induction.[12–21] These trials have demonstrated an improved remission rate for the newer drugs, although the recently completed MRC trial shows no benefit in the largest comparison of daunorubicin and mitoxantrone in remission rate, disease-free or overall survival, mitoxantrone did induce significantly more myelosuppression.[22] A direct comparison of the three drugs in an Eastern Cooperative Oncology Group (ECOG) study did not show any difference in remission rates.[23] The value of a third drug is controversial, but well established. The Australian Leukaemia Study Group evaluated the addition of etoposide as a third drug.[24] In younger patients only the long-term survival was better for the three drug arm (25% versus 17%). The UK MRC found no difference in any outcome measure in any subgroup when etoposide was compared with thioguanine as a third drug in a 10 day schedule.[1]

Further intensification of induction has been attempted by including high dose (3 g/m^2) Ara-C with daunorubicin and etoposide, in an Australian Leukaemia Study Group Trial.[25] There was no improvement in induction rate but disease-free survival (DFS) was significantly better in the high dose arm, although the overall survival was not different. A similar approach was evaluated by the Southwest Oncology Group (SWOG) using an Ara-C dose of 2 g/m^2.[26] Again no benefit was seen in remission rate in this study and although DFS was improved overall survival was not. The German AML Cooperative Group tested the concept of intervening with high dose Ara-C (3 g/m^2) as course 2 on day 21 of treatment. When compared with conventional dose treatment no overall differences were seen in CR, DFS or 5 year survival.[27] There was however benefit for patients who were defined as poor risk based on karyotype or having 50% blasts in the bone marrow on day 16 or a high serum lactate dehydrogenase. They had abetter remission rate, disease-free and overall survival. These high dose schedules were associated with increased toxicity, but they also provide some of the evidence that, while induction therapy may not change the induction rate, it may influence subsequent relapse risk. There is room therefore for improved regimens. Among the possibilities are reversal of chemoresistance (Chapter 12), intermediate dose Ara-C, antibody directed therapy or re-evaluation of anthracycline dose, some of which are being addressed in on-going trials.

PREVENTION OF RELAPSE

Most patients achieve remission, as defined, by conventional techniques. However, as previously discussed, recent more sensitive techniques clearly point to the fact that evidence of residual disease is common when the morphological criteria are met. The quality of 'depth' of remission may be quite variable in patients who enter remission but, to date, no robust studies have been able to quantitatively correlate the molecular

or other indicators of residual disease at the point of remission with the risk of subsequent relapse. It has long been established that consolidation of remission was desirable on the grounds – now confirmed by molecular evidence – that residual disease was present. The nature and number of courses required is an issue of current interest as the central question in several ongoing clinical trials. For the last 10–15 years adult patients under 60 years have been treated with one of three options. It is usual to establish the availability of an HLA matched sibling donor but, depending on transplant unit policy, this may be restricted to patients under 45 years. Patients who do not have a donor have been offered further intensive chemotherapy or high dose treatment with autologous stem cell rescue.

CHEMOTHERAPY

A number of studies have indicated that treatment given during remission induction, which may or may not shown differences in remission rates can influence disease-free survival.[25–27] Care must therefore be taken in evaluating post-remission or consolidation schedules in isolation without taking account of the efficacy of remission induction treatment.

HIGH DOSE ARA-C

A considerable evidence base has accumulated in recent years that demonstrates a dose–response relationship for Ara-C in AML. The studies in remission induction have already been discussed. Unfortunately plasma Ara-C pharmacokinetics does not correlate with intracellular drug concentration or clinical response.[28,29] The cellular uptake is related to plasma concentration. At lower Ara-C concentrations uptake is likely to be achieved by a transmembrane nucleoside transport system. Higher plasma concentrations may facilitate entry into the cell by additional diffusion mechanisms that may overcome the transmembrane process.[30] The intracellular conversion to Ara cytosine arabinoside triphosphate (CTP) by a sequence of kinases, is rate limited by deoxycytidine kinase, which reaches a peak in the mid and late S phase of the cell cycle.[31] Ara CTP is a potent inhibitor of DNA. Conventional dose Ara-C may be ineffective because of inadequate cellular uptake, reduced deoxycytidine kinase activity, rapid deamination on Ara-C or Ara cytosine arabinoside monophosphate (CMP), which prevents the formation of Ara CTP, increased catabolism of Ara CTP, or a low proportion of cells passing through S phase. Increasing the Ara-C dose can bypass at least some of these mechanisms.

Contemporary high dose Ara-C schedules are largely based on the preliminary studies by Herzig[32] who established the tolerability of a course of 3.0 g/m^2 every 12 hours for 12 doses (6 days). Central nervous system or gut toxicity were dose limiting. Several studies pursued this schedule with variable success, suggesting that it was only practical for patients less than 60 years. Some variation in schedules and number of courses used evolved around the 3 g/m^2 dose, in some cases in combination with other agents, most of which produced encouraging results.

The pivotal prospective study of Ara-C dose in consolidation was conducted by the Cancer and Leukemia Group B (CALGB) who compared three dose levels of Ara-C (100 mg/m^2/day by continuous infusion over 5 days versus 400 mg/m^2/day by continuous infusion over 5 days versus 3 g/m^2 intravenously twice a day on days 1, 3 and

5).[3] The high dose arm produced a superior event-free survival at 39% versus 21% and 25% in the lower dose arms. All patients were scheduled thereafter to receive four monthly cycles of Ara-C at conventional doses.

The protocol attempted to deliver four courses at the dose level allocated, but in practice only 56% received all four courses of the 3 g dose. Apart from compliance problems, dose escalation can be associated with extra toxicity, in particular cerebellar toxicity which appears to correlate with the patient's age, renal function and total dose given. In the CALGB study the toxicity in patients over 60 years became prohibitive.

A number of key issues need to be established with respect to Ara-C consolidation. The first is the issue of the dose given. It may well be possible to reduce toxicity and improve compliance with doses of Ara-C that are less than 3 mg/m^2, without reducing efficacy. Second is the question of the number of courses or days in each course. The study conducted by the Goelam Group[33], which was primarily designed to compare autologous bone marrow transplantation (BMT) with intensive chemotherapy (where the intensive chemotherapy induced a course of high dose Ara-C in a schedule of 3 g/m^2/12 hours for 4 days (24 g/m^2)), achieved a 43% and 59% disease-free and overall survival at 4 years, respectively. This suggests that as few as one course may be required. The superiority of the inclusion of a high dose Ara-C component was confirmed by an Easter Cooperative Oncology Group (ECOG) study that compared an arm with one high dose Ara-C course with a continuous (2 year) maintenance schedule. There was a trend in favour of the single high dose Ara-C arm, with DFS rates of 27% versus 16%, although the outcome was still not satisfactory.[34] Third is the question of whether high dose Ara-C in consolidation can be combined with high dose Ara-C in remission induction. This question was addressed in the Southwest Oncology Group (SWOG) study referred to earlier in which patients had received standard or high dose Ara-C in induction.[26] Those who entered remission were then eligible to be randomized to standard or high dose Ara-C in consolidation. In this study the patients induced with standard Ara-C showed no difference in survival or disease-free survival if they were randomized to standard or high dose Ara-C in induction. However the best outcome was seen in patients who were induced and consolidated with high dose Ara-C. However, that component of the study was not completely randomized so this was probably a selected group of patients and there was an inferior compliance with the treatment because of greater toxicity in induction. Any beneficial dose effect in this trial was limited to patients under 50 years of age.

It has been suggested that high dose Ara-C may be particularly effective in different prognostic risk groups. Patients in the CALGB Trial with t(8;21) disease had a particularly favourable outcome if given three or more courses of high dose Ara-C rather than one.[35] The number of patients was too small ($n = 21$) to be conclusive, and it needs to be borne in mind that intensive schedules not including high dose Ara-C can produce smaller survivals.[36] There is conflicting data as to the relative benefit of high dose Ara-C in poor risk patients with both favourable and unfavourable experiences. Acute promyelocytic leukaemia is a separate subgroup of AML that has long been known to have particular sensitivity to anthracyclines and, in recent years, to retinoic acid, combinations of which may make the use of Ara-C unnecessary.[37]

There are theoretical ways in which the sensitivity of leukaemic cells to Ara-C can be increased. Combination with fludarabine further enhances the pharmacology of Ara-C and this has been developed by the MD Anderson Group in particular in the fludarabine, Ara-C, G-CSF (FLAG) and FLAG-idarubicin (FLAG-IDA) regimens with encouraging results in AML and high risk myelodysplastic syndrome (MDS).[38] The contribution of granulocyte colony stimulating factor (G-CSF) or idarubicin is not

known and may not be substantial. This combination is well tolerated and effective but has never been subject to randomized comparison to other well established schedules. An on-going MRC trial is comparing fludarabine-Ara-C with an ADE (Ara-C, daunorubicin, etoposide) schedule for high risk or relapsed disease.

The second theoretical possibility is based on pre-clinical data suggesting that leukaemic blast cells can be made more sensitive to Ara-C following pre-treatment with retinoic acid.[39] The mechanism may be the shortening of the half-life of the anti-apoptotic protein Bcl-2.[40] Since Bcl-2 is frequently overexpressed in AML this may represent a mechanism of drug resistance that is amenable to intervention.[41] Small phase II studies have suggested clinical support for such a mechanism[42,43] but a randomized study in poor risk patients, although producing promising preliminary results, was not able to show a longer term benefit.[44,45] The current MRC AML12 trial is evaluating, based on the above rationale, the role of retinoid in non-APL patients when added to standard induction treatment but with two dose levels of Ara-C (200 mg/m^2/day for 10 days versus 400 mg/m^2/day for 10 days).

NUMBER OF CONSOLIDATION COURSES

It is clearly possible to reduce the intensity of each course of treatment and deliver several monthly treatments – even to the extent that it could be defined as maintenance therapy. An MRC trial addressed the question of eight versus four courses and was not able to show any benefit for the additional courses, which has been confirmed by the Finnish Group who compared a total of four versus eight courses, and this could also be observed in the older patients.[46,47] In the last decade the trend has been to intensify each course of treatment and rely on improved supportive care to protect the patient. This has resulted in cumulative haematological toxicity and has restricted the compliance achievable with more than two or three courses of consolidation treatment. This may be compounded by the use of more myelotoxic combinations during induction treatment. The MRC10 Trial, which will be discussed in more detail below, was primarily designed to evaluate the role of transplantation when added to a total of four courses of treatment – which for most patients meant three consolidation courses. A general conclusion from that large trial which recruited nearly 2000 patients was that more, i.e. the addition of a transplant, was better in terms of prevention of relapse. The current follow-up trial designated AML12 tests the value of a total of five versus four courses of total treatment.

ALLOGENEIC TRANSPLANTATION

The transition of allogeneic transplantation from an experimental treatment to standard care in the early 1980s was of particular value in AML when 70–80% of patients who achieved remission relapsed when given what was at that time thought to be the best available chemotherapy. Overall, durable survivals of around 50% were reported with a risk of relapse of 15–20% if a transplant was applied in first remission. Prospective comparative studies at the time confirmed an overall survival advantage for allograft even allowing for the fact that patients who actually received the transplant were already selected because they had avoided relapse or the rigours of prior treatment and were considered fit enough to undergo transplantation. Approximately 20–30% of patients died after transplantation for reasons other than relapse. These included

graft-versus-host disease (GVHD), cytomegalovirus (CMV) disease, infections etc. In the intervening 15–20 years huge efforts have been made in the field of bone marrow transplantation, in general aimed at eliminating these transplant related complications, and thus enabling the anti-leukaemic mechanisms to fulfil their potential. While progress has been made in the individual components these have frequently been at a price, with the current situation being that allografting still carries a procedural mortality of 15–20%. CMV disease and pneumonitis has been substantially reduced by the use of CMV negative blood products, in those circumstances where the donor and host are CMV negative, and the effective and prophylactic use of anti-viral agents in high risk CMV seropositive patients as well as the use of more precise diagnostic methods. Efforts to prevent GVHD by ex vivo T depletion of the graft were highly successful but in some, although not all, series this led to an increased relapse rate in AML highlighting what was well known to the pioneers of transplantation, i.e. that the graft could exert an anti-leukaemic effect. The graft versus leukaemia (GVL) effect is a major factor in disease control in AML but evidence from T depletion series and the experience of donor lymphocyte infusion in relapsed disease do not suggest as powerful an effect in AML as in chronic myeloid leukaemia. There appears to be little to choose between standard preparative regimens e.g. cyclophosphamide combined with either total body irradiation (TBI) or busulphan. However, there is a dose relationship with TBI. In a randomized comparison of 15.75 Gy versus 1.2 Gy the Seattle Team demonstrated a reduced relapse risk with the higher dose but this was offset by increased toxicity. This provides a rationale for novel ways of delivering irradiation (see Chapter 12).

The classic relationship between acute and chronic GVHD on relapse risk in leukaemia was not demonstrated in the subgroup of patients with AML in first remission. Other data suggests that any relationship is more likely to be associated with chronic GVHD. The degree of immunosuppression after transplant may be directly related to relapse risk.

A lot has been learned about the treatment of AML from the transplant experience, not least in the area of supportive care. This has enabled more intensive chemotherapy to be delivered in relative safety. It also provides a stimulus for the development of immune mediated techniques of disease control. Peripheral blood cells from the donor are likely to provide future opportunities for improvement.[48,49] It appears that results are improved with a larger cell inoculum and that immune and haematological reconstitution is more rapid. Little is yet known about disease control but preliminary data is encouraging. It is important to bear in mind that only a minority of patients with AML are eligible for this option because of donor availability or on the grounds of age. There is relatively little experience of HLA-matched unrelated transplantation in AML. One prospective study tracked patients who were potentially eligible for allograft from diagnosis and highlighted the fact that many patients did not progress to allogeneic transplant, implying that those who do are selected. This provides the strong argument of using an intent-to-treat or donor-versus-no donor approach to prospective studies comparing transplant with chemotherapy, although this is not unanimously accepted.

AUTOLOGOUS TRANSPLANTATION

On the assumption that the myeloablative treatment component of allogeneic transplantation was a major component of the anti-leukaemic effect of allogeneic BMT, and secure in the knowledge that cryopreserved marrow collected in remission could regenerate haematopoiesis after TBI, several groups conducted unrandomized studies

of autologous BMT as consolidation of first remission.[50–55] The results were encouraging with 45–55% of patients surviving. Unlike allografting most patients failed because of relapse with non-leukaemic deaths occurring in 6–8% of recipients.

Some groups considered that it was almost unethical to reinfuse untreated marrow which, because most patients would otherwise be expected to relapse, had a high likelihood of leukaemic contamination if no effort was made to eliminate occult leukaemia. The most widely adopted purging method was pharmacological using the cyclophosphamide derivative 4-hydroxypercyclophosphamide.[56] This was chosen because it had proved effective in a rat model system that was however very sensitive to cyclophosphamide. Pioneering studies in Baltimore established an in vitro dose that was compatible with haematological recovery.[57] In phase II clinical trial in second CR using busulphan and cyclophosphamide as conditioning, promising results were observed. Similar data was produced in CR2 using busulphan/cyclophosphamide and unpurged marrow, thus raising doubts about the role played by purging.[58] In Europe considerable enthusiasm was generated by the Paris group who, consistently, from their own institution or through the European Group for Bone Marrow Transplantation (EBMT) registry, demonstrated good results in recipients of purged marrow.[55,59] The criticism has always been that unconscious patient selection pressures could have been in play. In the EBMT registry data it is suggested that patients at higher risk from relapse might benefit most from ex vivo purging. These were defined as patients initially entering CR late – i.e. >40 days – or having the autograft early in remission.[55] The difference in outcome for these patients in registry data is such that a comparative study could be satisfactorily carried out with reasonable patient numbers.

CONTEMPORARY CLINICAL TRIALS

The prognosis for adult patients with AML under 60 years has improved substantially in the last 15 years (Figure 1). It is therefore appropriate that in recent years

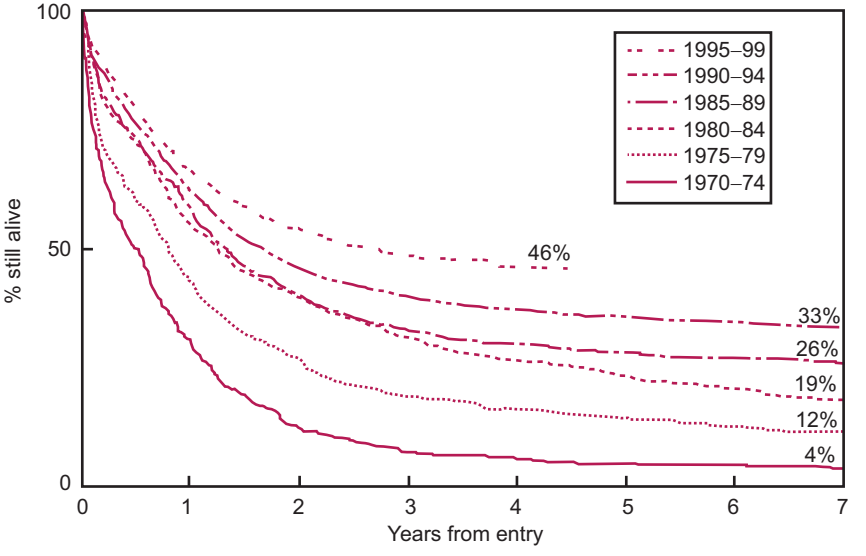


Figure 1. Improved survival in patients aged 15–59 years who entered MRC Trials in 1970–1999.

Table I. Recent trials evaluating allogeneic transplant on a donor versus no donor basis.

	Disease free survival (% at 4 years or beyond)			Overall survival (% at 4 years or beyond)		
Trial	Allograft		Chemotherapy	Allograft		Chemotherapy
EORTC-GIEMEMA 295 vs 377†[70]	46	vs	33**	48	vs	40
GOELAM 88 vs 157[33]	44	vs	38	53	vs	53
MRC 428 vs 870[61,63]	49	vs	42	55	vs	50
US Intergroup 113 vs 117	43	vs	35	46	vs	52

†Number of patients: allograft (i.e. donor available) versus chemotherapy. $^{**}P = 0.01$.
Abbreviations used: EORTC-GIEMEMA, European Organisation for the Research and Treatment of Cancer – Gruppo Italiano Malaltie Ematologiche Maligne dell'Adulto; GOELAM, Groupe Quest Est Leucémies Aiguës Myéloblastiques; MRC, Medical Research Council; vs, versus.

comparative studies have been undertaken to re-evaluate the relative benefits of allogeneic and autologous transplantation, particularly since the selection pressures favouring those who undertook transplantation were better defined.[60]

Major collaborative groups such as EORTC-GIEMEMA, UK MRC, GOELAM, HOVON, and the US Intergroup all addressed the issue in studies in adults spanning the last 10 years.[2,33,54,61,62] All conducted large prospective trials that were primarily addressing the role of autologous transplantation as consolidation versus chemotherapy (EORTC-GIEMEMA, GOELAM, US Intergroup) or in addition to chemotherapy (MRC[63], HOVON). In patients where a donor was identified, allogeneic BMT was recommended and thus a comparison with chemotherapy was possible. Since patients were not randomized the comparison was available on a donor versus no donor basis as a surrogate for 'intention-to-treat' with all patients having a donor available being regarded as transplant recipients. The results of these endeavours are shown in Table I. Based on this analysis all studies showed a reduction in relapse risk, confirming the superiority of the mechanisms of transplantation in preventing relapse compared with chemotherapy. However this did not convert to an overall survival advantage in any study. Interim data from the MRC10 trial endorsed the requirement to evaluate the role of allograft on a donor versus no donor basis. When patients who received an allograft were compared with those who did not have a donor and were therefore treated only with four courses of chemotherapy, they appeared to have a survival advantage. However, the survival of patients who had a donor available but did not receive the transplant – and who therefore received the same four courses of chemotherapy only – was not only inferior to the survival of patients who received an allograft, but also to the no donor group. These data strongly infer that the patients who actually received the transplant were selected to be at better risk since patients with a poorer prognosis did not get to the transplant. This form of analysis does not find favour in all quarters[64], but when contemporary studies are analysed on a donor versus no donor basis the advantage of transplant is less clear in spite of confirmation of its powerful anti-leukaemic effect.

PROSPECTIVE TRIALS OF AUTOLOGOUS BMT

Four large trials attempted to prospectively compare autologous BMT with intensive chemotherapy in adults. Three compared autograft versus chemotherapy. These

include the EORTC-GIEMEMA, GOELAM and US Intergroup studies[2,33,62], which in total recruited 2230 patients and randomized 629. All indicated that there were fewer relapses in the autograft arm. Only the EORTC trial showed an improved disease-free survival, but none showed an overall survival advantage (Table 2). Each trial has particular points of interest. The reduced risk of relapse is apparent in all three in spite of the fact that the compliance with autograft was between 54–87%. In this respect the anti-leukaemic potential may be underestimated.

The outcome in the chemotherapy arm of the EORTC trial was less than is currently achievable for other contemporary schedules and therefore explains the difference in disease-free survival. The valuable feature of this study is that it demonstrates that patients who relapse from chemotherapy can be rescued by a transplant in CR2, thus delivering on equivalent overall survival. This approach as an elective strategy will be discussed below. Both the GOELAM and US Intergroup trials clearly delivered a better chemotherapy arm and it is of interest that in both cases this included high-dose Ara-C. The GOELAM trial is in many ways the most complete study because the randomization efficiency and compliance with allocated treatment was superior to other studies. The chemotherapy arm survival was 55% and thus explains why no advantage could be demonstrated for autograft overall. The US Intergroup trial had a poor compliance with allocated treatment and has emphasized the problem of treatment delivery. It was possible to administer the intended high dose Ara-C to a high proportion of patients without delay, whereas compliance with autograft was poorer partly because of delay in delivery, which contributed to poorer compliance due to intervening relapse. The MRC and unpublished HOVON trials posed a different question, namely the role of autograft in addition to what was considered to be adequate prior chemotherapy – in other words to deliver myeloablative therapy at a time when residual disease might already be minimal. The MRC study[61], like other trials, demonstrated a major reduction in relapse risk which is particularly important since one-third of patients did not receive the autograft and might under-represent its anti-leukaemic potential. Prior relapse was not a major reason for non-compliance. This benefit did convert into a significant improvement in disease-free survival but the overall survival advantage only became apparent after 2 years of follow-up when the competing effects of excess deaths and improved leukaemia control stabilized. On the intent-to-treat analysis in this study there were excess deaths in the autograft arm (15%). This and the compliance rate of 65% are partly explained by the trial design where patients were randomized after three courses and then all had to receive a fourth course prior to autograft. This provided an additional interval of danger for patients, particularly as the period of haemopoietic recovery became progressively longer, to become clinically unfit. Of those patients who received the autograft, 11% died without leukaemia. In none of these prospective trials was the result achieved by autograft inferior to that predicted in the earlier single arm studies. Purging was only used in the US Intergroup trial and did not appear to be associated with a reduced relapse risk. The autograft mortality varied between trials but was generally higher than that reported from single institution studies. This might be related to the tendency to give more intensive prior chemotherapy in contemporary practice.

POTENTIAL FOR IMPROVING THE AUTOGRAFT APPROACH

Careful prospective trials have not provided convincing evidence that autograft is superior to intensive chemotherapy with high dose Ara-C but it is clearly a superior

Table 2. Prospective trials of autologous transplant in adults.

	Relapse (% at 4 years or beyond)			Disease-free survival (% at 4 years or beyond)			Overall survival (% at 4 years or beyond)		
Trial	Autograft		Chemotherapy	Autograft		Chemotherapy	Autograft		Chemotherapy
EORTC-GIEMEMA 115 vs 117†[2]	40	vs	57	48	vs	30*	56	vs	46
GOELAM 86 vs 78[33]	NA			44	vs	40	50	vs	55
MRC 190 vs 191[61]	37	vs	58**	53	vs	40***	57	vs	45
US Intergroup 116 vs 117[62]	62	vs	48	35	vs	35	43	vs	52*

†Number of patients randomized to autograft vs chemotherapy. *$P = 0.05$; **$P = 0.0007$; ***$P = 0.04$, NA not available; vs, versus. For details of trials see Table 1.

Table 3. Compliance with randomization and allocated treatment in autograft trials.

Trial	Patient number	CR%	% of remission patients randomized	Number randomized	% randomized who received autograft
EORTC-GIEMEMA[2]	941	66	63	232	71
GOELAM[33]	517	71	61	164	87
MRC[1]	1966	80	34	381	66
US Intergroup[62]	772	70	60	233	54

CR, complete remission. For details of trials see Table 1.

anti-leukaemic modality. The failure to translate this into an overall survival advantage was due to the associated treatment mortality and the observation – particularly demonstrated in the EORTC trial – that some patients who relapse can still be salvaged. A further major issue is that of deliverability of the procedure. As all studies have shown only a minority of patients who were eligible for autograft received it (Table 3). The major reason for autograft failure is relapse.

AUTOGRAFT SAFETY

Approximately 10% of patients undergoing autograft will die in remission. This still represents a small number of events in the total trial experience and it is not possible to clearly identify single reasons. In the MRC experience deaths tended to be associated with poor engraftment. Protracted thrombocytopenia seems to be a feature of AML autografts. In a retrospective analysis, platelet count at the time of stem cell harvest emerged as the main associated predictive variable.[65] No studies using thrombopoietic growth factors have been conducted. In an attempt to identify poor engrafters we conducted a retrospective analysis of the haemopoietic potential of the stored graft using a long-term culture system. The endpoint was the ability of the culture to continue to generate CFU-GM into the supernatant for more than 4 weeks. There was no relationship between 'good' and 'poor' growers with respect to haemopoietic regeneration post-autograft, but there was a significantly better leukaemia-free survival in good (65%) versus poor (18%) growers.[66]

In the MRC10 trial all patients had to have bone marrow harvested before they were randomized, with a recommendation that at least 1.0×10^8 mononuclear cells/kg were stored. A range of doses were collected. In recipients of autograft there was no clear relationship between mononuclear cell dose given and neutrophil or platelet regeneration. However, the cell dose collected had a strong relationship to relapse and survival in the autograft arm either when analysed on an intent-to-treat or on an autograft-received basis. The harvested dose was not in itself a prognostic factor for disease outcome because there was no predictive value in patients in the chemotherapy arm. Peripheral blood (PB) represents a more attractive source of stem cells. When added to bone marrow there was clear improvement in recovery of both neutrophils and platelets. Registry data where PB was the only source of stem cells suggests a more rapid recovery of neutrophils, but that for some patients platelet recovery remains slow. Whether collection of stem cells is possible in all eligible patients is not yet known. The current MRC12 trial gives investigators the option to collect PB cells on recovery from the second or third courses: 75% of patients were successfully collected with a single

procedure after course 2 and 50% after course 3.[67] Adequate collections may be less feasible after more than three courses and a successful collection might indicate a favourable prognosis. These important issues may be resolved by the recently concluded EORTC10 trial, which prospectively compared bone marrow and peripheral blood autografts in a randomized fashion in AML in first remission.

The EORTC trial raised the question of whether all patients should be harvested in first remission but the autograft delayed until treatment failure. This is feasible for some patients, but whether these patients can be identified is less clear and will be discussed below. Whether patients need to be returned to remission before autografting is worthy of consideration. Autografting in treated relapse, i.e. when reinduction therapy has failed, is discredited. Attempts at achieving a second remission will fail in about 50% of patients, although the risk may vary widely between patients and has relatively little to do with the reinduction protocol used. Using autograft as the initial treatment of relapse has the advantage of delivering the procedure to all the patients but will be associated with an increased risk of relapse. However, the number of overall cures achieved may not be inferior to the more widely used approach of inducing CR and deploying transplant as consolidation. This strategy has been tested only in one study and clearly depends on the ability to deliver the autograft to relapsed patients at short notice.[68]

Deliverability

A major restriction of autograft that has emerged from the prospective trials is that only a minority of potentially eligible patients were randomized in the trial (34–63%) and the allocated treatment was given in 54–87% of cases (Table 3). So the proportion of eligible patients who could have received this treatment was low (22–53%). This casts doubt on the point of having an effective treatment if it is only deliverable to a minority of patients. This is a flaw of trial design. Original registry data demonstrated that the relapse risk was greater if patients were autografted early in remission. This is partly explained by the inclusion of patients who are at a higher risk of relapse and who do not stay in remission until a later autograft – although it is of interest that there was little evidence of this in the MRC10 trial where the same proportion of good, standard and poor risk patients which entered CR were randomized. It was assumed that consolidation therapy was essential to success by effecting 'in vivo' purging. With more intensive approaches to remission induction therapy it may be reasonable to re-examine the option of delivering the autograft earlier in remission and aiming to include more eligible patients.

A RISK DIRECTED APPROACH

Prospective comparisons of the three treatment options suggest that overall these treatments produce equivalent survival. Transplantation is expensive but whether it is more expensive than effective chemotherapy is an open question. Recipients of chemotherapy have a poorer quality of life and inferior sexual health than the recipients of transplantation, with allografting having a greater impact than autografting. It is very clear in a heterogeneous disease like AML that there is a considerable variation in relapse risk in patients given identical treatment. This has enabled a range of risk factors to be identified, which are independently predictive and remain predictive when applied to other datasets (see Chapter 4). There is much interest in

using some of these factors to categorize different risk groups and then analyse the treatment options by risk group. This is already built into some current clinical trials and is likely to become standard practice. The most obvious example of this approach is the separation of acute promyelocytic leukaemia on the basis of its sensitivity to retinoic acid and anthracyclines for a separate treatment approach (see Chapter 9).

The recent prospective trials have provided a useful opportunity to analyse results based on the patient's risk profile. Each trial group has used different definitions (Table 4) of relapse risk, which are sometimes derived from the same dataset and have the limitation that they have not been prospectively validated. Using cytogenetic definitions of risk it is clear that risk group dictates outcome irrespective of treatment option used.

An analysis of the US Intergroup trial based only on cytogenetics has been presented.[69] The MRC adopted a similar cytogenetic subgroup definition but also included in the poor risk category patients who failed to clear more than 85% of blast cells with the first treatment course unless the chromosomes were favourable, which has been prospectively validated in the current MRC AML12 trial.[9,22] The EORTC-GIEMEMA and GOELAM trials had insufficient cytogenetic data available and used a combination of French-American-British (FAB) group, presenting white blood cell (WBC) count and achievement of remission in the first course to devise three risk groups.[33,70] To establish whether these risk definitions were equivalent they were applied to the MRC10 dataset (G Harrison, pers. comm.) (Figure 2). The cytogenetic based definitions produced identical results, whereas the other definitions produced less good, but significant, discrimination.

GOOD RISK PATIENTS

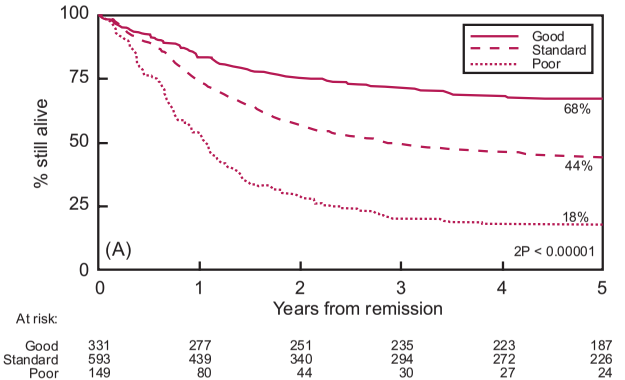
Based on a donor versus no donor analysis within the good risk definition used by the individual trial groups, there was a trend that was significant in the EORTC trial for a reduced relapse risk in the donor arm, but no general benefit in disease-free survival at 5 years (Table 5). Three trials showed no survival benefit in the donor arm. However, the donor arm was superior in the US Intergroup Study, but this must be viewed with caution, first because the numbers are small, second because only 35% of good risk patients in the chemotherapy arm survived (suggesting a chance finding), and third because it appears inconsistent with other trials particularly with the MRC trial where the risk group definitions were similar. Less information is available concerning autografting (Table 6). In the MRC experience the addition of autograft significantly reduced the relapse risk and improved the disease-free but not the overall survival because patients in this category who relapsed could be salvaged. Again the US Intergroup showed a superior survival largely because of the poor outcome on the chemotherapy arm. Although not strictly comparable allograft and autograft resulted in equivalent survival.

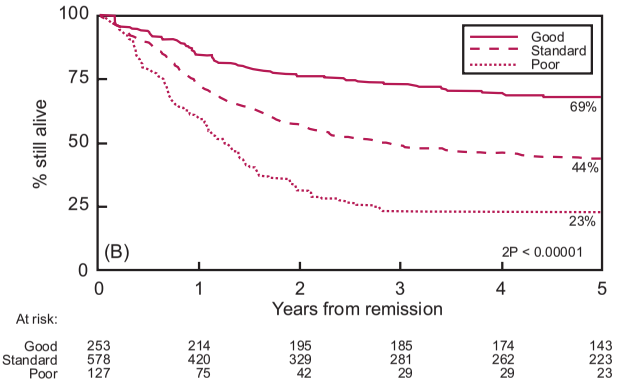
Given the equivalence of overall survival in three out of four trials, transplantation in first remission appears unnecessary particularly when the data on quality of life, fertility and sexual health and, in children, intracellectual development and growth retardation are borne in mind. However, the reduction in relapse risk demonstrated in the MRC trial shows that more treatment has the potential to improve disease control in this subgroup, which is currently being subjected to a randomized comparison of four versus five courses of total therapy in the MRC AML12 trial. This is the subgroup who may benefit particularly from high dose Ara-C.[35]

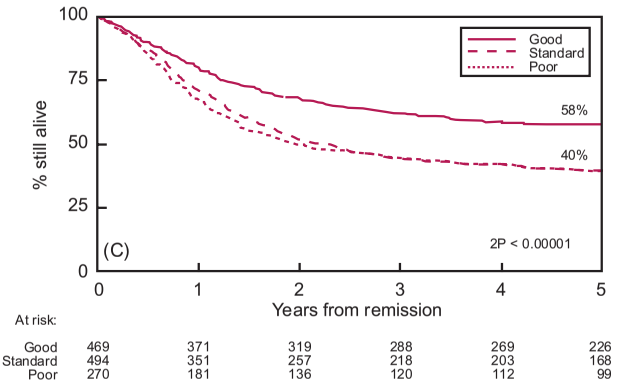
Table 4. Risk group definition used by transplant trial groups.

	MRC	US Intergroup	EORTC	GOELAM
Good	M3, t(15;17) t(8;21) inv(16)	t(15;17) with other: inv(16) t(8;21) without del(9q) or complex	−CR 1st course +FAB M2/M3/M4e −M1/M4 + WBC < 25	M2,M3 + WBC < 30
Standard	Not good or poor	+8, −Y, +6, del(12p) normal	−CR 1st course with unfavourable FAB or WBC > 25 −CR > 1st course with favourable FAB + WBC < 25	M0,1,2,4,5,6,7 and WBC > 30
Poor	−5/del(5q); del(7q), 3q−, complex	−5/del(5q), −7/del(7q), inv(3q), 11q, 20q, 21q, 17p del(9q), t(6;9), t(9;22) complex	−CR > 1st course FAB 5,6,7 −M1,2,3,4, + WBC > 25	M0,1,2,4,5,6,7, and WBC < 30

For details of trials see Table 1.
Abbreviations used: CR, complete remission; FAB, French-American-British; WBC, white blood cell count.







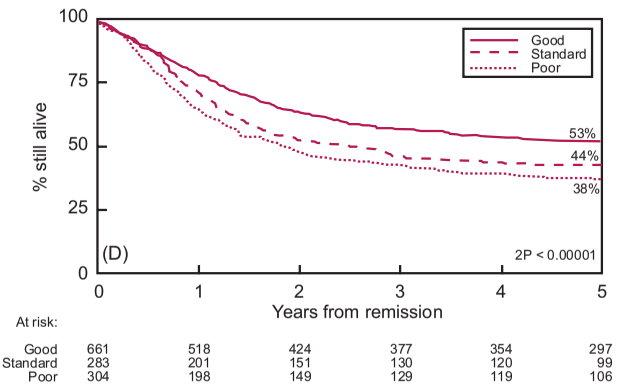


Figure 2. Assessment of different risk group definitions against MRC AML 10 database. **A**, Survival from complete remission (CR) by MRC risk group. **B**, Survival from CR by US Intergroup risk group. **C**, Survival from CR by GOELAM risk group. **D**, Survival from CR by EORTC risk group. The risk groups used by each trial group are given in Table 4, and were applied to 1500 patients in the MRC10 database.

Table 5. Transplant versus chemotherapy in prospective trials: good risk.

	GOELAM donor/ no donor	MRC donor/ no donor	EORTC donor/ no donor	US Intergroup donor/no donor
Number	35/39	117/227	129/155	19/20
Relapse (%)†	NA	26/36	30/49*	NA
Disease-free survival (%)†	61/51	61/60	57/45	NA
Survival (%)†	71/67	71/73	61/56	66/35

For details of trials see Table 1.
†At 5 years.
*$P = 0.01$; NA, not available.

Table 6. Transplant versus chemotherapy in prospective trials: good risk.

	GOELAM auto/chemo	MRC auto/chemo	EORTC auto/chemo	US Intergroup auto/chemo
Number	32/22	51/44	NA	21/20
Relapse (%)†	NA	25/49*	NA	NA
Disease-free survival (%)†	50/57	70/48**	NA	NA
Survival (%)†	59/71	74/61	NA	74/35‡

For details of trials see Table 1.
†At 5 years.
*$P = 0.02$; **$P = 0.04$; ‡ no P value available; NA, not available; auto, autologous bone marrow transplant; chemo, chemotherapy.

Table 7. Transplant versus chemotherapy in prospective trials: poor risk.

	GOELAM donor/ no donor	MRC donor/ no donor	EORTC donor/ no donor	US Intergroup donor/no donor
Number	22/32	51/110	46/69	18/22
Relapse (%)†	NA	71/78	69/87*	NA
Disease-free survival (%)†	27/22	22/21	22/12	NA
Survival (%)†	41/30	23/25	28/22	42/15‡

For details of trials see Table 1.
†At 5 years.
‡No P value available.
Donor versus no donor: *$P = 0.03$.
NA, not available.

Table 8. Transplant versus chemotherapy in prospective trials: poor risk.

	GOELAM auto/chemo	MRC auto/chemo	EORTC auto/chemo	US Intergroup auto/chemo
Number	21/22	22/20	NA	25/22
Relapse (%)†	NA	56/73	NA	NA
Disease-free survival (%)†	38/29	44/27	NA	NA
Survival (%)†	47/40	49/39	NA	NA

For details of trials see Table 1.
†At 5 years.
NA, not available, auto, autologous bone marrow transplantation; chemo; chemotherapy.

POOR RISK PATIENTS

Poor risk patients under 60 years are a minority population so the numbers available within the trials has been small. Neither the GOELAM, MRC or EORTC trials could demonstrate a benefit for allo or auto transplant (Tables 7 and 8). The Intergroup trial, while showing no advantage for autograft showed a superior survival for allogeneic transplant of 42% versus 15% at 5 years. In the absence of improved chemotherapy in this difficult patient group allogeneic transplant, probably including matched non-sibling donors seems justifiable. The results may be improved if transplantation is undertaken as soon as a patient is defined as poor risk. However, the relapse risk will continue to be high and novel approaches to reducing relapse risk are needed, such as pre-emptive donor lymphocyte infusion. These patients are functionally chemoresistant and are a target group for experimental approaches aimed at overcoming resistance (see Chapter 12). There is conflicting data as to whether these patients benefit from high dose Ara-C or not.

STANDARD RISK DISEASE

Most patients are at standard risk of relapse. The benefits of allograft on a donor versus no donor analysis were only seen in the MRC trial with a non-significant trend for superior survival in the donor arm in the GEOLAM and US Intergroup trials (Table 9). A favourable impact on relapse risk where it was stated, was seen in both the MRC and EORTC trials. No overall survival benefit for autograft was seen in any of the four trials (Table 10).

Table 9. Transplant versus chemotherapy in prospective trials: standard risk.

	GOELAM donor/ no donor	MRC donor/ no donor	EORTC donor/ no donor	Us Intergroup donor/no donor
Number	31/63	194/428	100/130	47/44
Relapse (%)†	NA	34/56[1]	47/66[2]	NA
Disease-free survival (%)†	34/38	53/39[3]	42/29	NA
Survival (%)†	41/57	57/45[4]	46/38	52/55

For details of trials see Table 1.
†At 5 years.
[1]$P = 0.00002$; [2]$P = 0.05$; [3]$P = 0.003$; [4]$P = 0.02$; NA, not available.

Table 10. Transplant versus chemotherapy in prospective trials: standard risk.

	GOELAM auto/chemo	MRC auto/chemo	EORTC auto/chemo	Us Intergroup auto/chemo
Number	33/35	97/112	NA	37/44
Relapse (%)†	NA	40/59*	NA	NA
Disease-free survival (%)†	39/31	49/39	NA	NA
Survival (%)†	43/51	52/40	NA	36/55

For details of trials see Table 1.
†At 5 years.
*$P = 0.05$; NA, not available; auto, autologous bone marrow transplantation; chemo, chemotherapy.

The danger of subgroup analysis is well known, and the conclusions within subgroups are therefore to be treated with caution. The data when analysed by risk group provides no convincing support to routinely offer transplant to all patients in CR1 as standard care. It is reasonable within the context of a clinical trial to continue to address the question of transplant, particularly as it appears that the trend for improved outcome for chemotherapy with time continues, and when peripheral blood allograft holds promise.

MINIMAL RESIDUAL DETECTION

While several factors have been identified that predict relapse risk, there is much interest in applying novel laboratory techniques to detect residual disease. Conventional metaphase cytogenetics and fluorescent in situ hybridization (FISH) on interphase cells are not sensitive enough to be useful. However, there are few studies prospectively evaluating sequential monitoring by FISH with clinical outcome correlations. Immunological characterization of leukaemic blasts at diagnosis using an extensive panel of antibodies can delineate leukaemic phenotypes that are not represented in normal haemopoiesis. Detection of these phenotypes in remission marrow is possible at a level of 1×10^5 or 1×10^6, but considerable expertise is required to interpret the flow cytometry patterns. Preliminary studies confirm the utility of this approach.[71]

Reverse transcriptase polymerase chain reaction (RT-PCR) studies have been most extensively established in APL. Techniques developed to a sensitivity of 1×10^5 or 1×10^6 confirm detectable 'disease' in patients in long-term remission.[72] Studies with sensitivities of 1×10^4 have had more clinical relevance. RT-PCR detection of the PML-RARα transcript with that level of sensitivity is now accepted as being predictive of relapse.[5,6,73] Patients who, after consolidation, remain positive or who have been RT-PCR negative and become positive, are at very high risk of relapse. The apparent inevitability of haematological relapse following RT-PCR positivity has, for several groups, justified therapeutic intervention, without waiting for relapse. There is much less similar information available for other molecularly accessible diseases such as t(8;21) and particularly inv16.[74] A negative RT-PCR at an early stage of treatment appears to predict for a low relapse risk.[7] Transcript quantification by techniques such as real time PCR are theoretically attractive for relating residual disease to relapse risk more precisely. However, preliminary studies directly comparing this approach with a single RT-PCR at a designated time point did not demonstrate any increased sensitivity.[75] This technology has great potential to assist clinical decision-making, but care and patience are required to validate the clinical applicability. The approach assumes that detection of the transcript means residual disease and that a marrow or blood sample is representative of the distribution of disease as a whole. It is also conceivable that the significance of the molecular data may be different for each genetic lesion.

TREATMENT OF RELAPSE

In spite of the undoubted improvement in disease control by the adoption of a more intensive approach to first line treatment, the majority of patients will relapse. It has been recognized for some time that the profile of the relapsed patient is more influential on subsequent outcome than the treatment given. In general, age and

Table 11. Outcome after relapse by risk group.

	Risk group				Age Group		
	Good	Standard	Poor	Unknown	0–14	15–34	>34
Second remission (%)	90	54	45	45	65	48	62
Survival from relapse at 2 years (%)	38	9	15	9	35	16	12

Patients relapsed from chemotherapy alone in the MRC AML10 Trial (Burnett et al. 1998[61]).

length of first remission will predict the likelihood of achieving another remission. Older patients with short first remissions have a low prospect of responding to further treatment, while younger patients with CRI duration beyond 1 year should certainly have further attempts at reinduction. Even though more intensive first line treatment is now given, the relationships of age and CR1 duration remain valid, and have been confirmed on a large database. Second remission achievement and duration are also related to cytogenetic risk group (Table 11). These data are valuable when assessing the impact of treatment or advising a relapsed patient.

There is no standard treatment for relapsed disease. If a transplant can be given in second remission about 25–35% of patients can be salvaged, but the duration of second remission prior to transplant can dramatically influence this prediction because of the effects of time censoring which is much more pronounced in second CR than in first CR. Several schedules have claimed to be superior to others in this setting but randomized comparisons are scarce. The MRC compared a standard ADE schedule with the sequential ADE schedule shown to be efficacious by the GOELAM group.[76,77] Overall patients did poorly although standard ADE emerged as superior with respect to remission rate, but there was no difference in disease-free or overall survival. Patients who relapse have a high probability of having an multi-drug resistance (MDR) phenotype, in particular P glycoprotein (Pgp) overexpression. Overcoming resistance will be discussed in detail in another chapter, but the MRC relapse trial attempted to modulate the daunorubicin with cyclosporine without showing any benefit. It is probable that the 5 or 10 mg/m^2 cyclosporine doses used were inadequate. In a SWOG study a cyclosporine dose of 15 mg/m^2 given with continuous infusion of daunorubicin in a (7 + 3 day) schedule was superior to D + A alone.[78] Relapse is an appropriate setting for evaluating new agents but it is important that patients are well characterized by risk factors. Salvaging patients with good risk features is realistic, but for other risk groups the priority must be to improve first line treatment. No studies have compared consolidation treatments in CR2. It is standard practice to offer allograft or autologous transplant. In an attempt to compare allograft with chemotherapy a retrospective analysis on International Bone Marrow Transplant Registry (IBMTR), MRC and German Trial Group data was compared.[79] Overall leukaemia-free survival was better for transplant (26% versus 17%). Age (greater or lesser than 30 years) and CR1 duration (greater or lesser than 12 months) were major predictive factors for survival. When the datasets were compared within the risk stratifications, the benefit of transplant was clearer for younger patients in remissions of >1 year (41% versus 17%) and to a lesser extent in older patients with short first remissions (18% versus 7%). Other subgroups had similar survival with each treatment approach.

FUTURE PROSPECTS

The improved survival in younger patients with AML observed in recent years has been encouraging and is probably attributable to improvements in supportive care that have enabled a more intensive treatment approach to be adopted. Ara-C has a crucial role to play and more careful evaluation of dose and scheduling is worthy of prospective study. Available chemotherapy is now delivering good results in good risk patients such that transplantation is not required as first line treatment. It is however failing poor risk patients and novel approaches are required for these patients who have primarily resistant disease. There is some evidence to suggest that more treatment may benefit standard risk patients who are the majority. Progress will be made in developing more accurate risk direct treatment. Antibody targeted treatment (Chapter 11) may enable more treatment to be given with minimal additional collateral damage.[80] As well as using prognostic factors such as cytogenetics to tailor treatment, more comprehensive use of molecular and possibly immunological techniques to predict the volume of residual disease will enable targeting of extra treatment or transplant appropriately. As chemotherapy improves conventional allo or autografting may be difficult to justify in any patient, but could be reserved for relapse or other high risk situations only. The use of allogeneic peripheral blood stem cells as the source of stem cells may redefine the role of transplant[48,49], but this will still require assessment in comparative studies.

Different treatment approaches may be required for different AML subgroups. This will create major difficulties for traditional randomized trials because no collaborative group will attract sufficient patients. Either novel statistical approaches will be required or major international collaboration will need to be established to ensure that potential improved treatments are properly validated.

Acknowledgements

The author is grateful to Georgina Harrison and Keith Wheatley for analysing the AML10 database with different risk definitions and to Siân Edwards for preparing the manuscript.

REFERENCES

1. Hann IM, Stevens RF, Goldstone AH et al. Randomized comparison of DAT versus ADE as induction chemotherapy in children and younger adults with acute myeloid leukaemia. Results of the Medical Research Council's 10th AMI Trial (MRC AML 10). *Blood* 1997; **89:** 2311–2318.
* 2. Zittoun R, Suciu S, Watson M et al. Quality of life in patients with acute myelogenous leukemia in prolonged first complete remission after bone marrow transplantation (allogeneic or autologous) or chemotherapy: a cross-sectional study of the EORTC-GIEMEMA AML 8A trial. *Bone Marrow Transplantation* 1997; **20:** 307–315.
* 3. Mayer RJ, Davis RB, Schiffer CA et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *New England Journal of Medicine* 1994; **331:** 896–903.
4. Cheson BD, Cassileth PA, Head DR et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *Journal of Clinical Oncology* 1990; **8:** 813–819. (Review).
5. Lo Coco F, Diverio D, Avvisati G et al. Molecular evaluation of residual disease as a predictor of relapse in acute promyelocytic leukaemia. *Lancet* 1992; **340:** 1437–1438.
6. Burnett AK, Grimwade D, Solomon E, Wheatley K & Goldstone AH. Presenting white cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukaemia treated with all-trans retinoic acid: result of the randomised MRC trial. *Blood* 1999; **93:** 4131–4143.

7. Morschhauser F, Cayuela JM, Martini S 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. *Journal of Clinical Oncology* 2000; **18:** 788–794.
8. Gale RE & Linch DC. Clonality studies in acute myeloid leukemia. *Leukemia* 1998; **12:** 117–120.
* 9. Wheatley K, Burnett AK, Goldstone AH et al. A simple robust and highly predictive prognostic index for the determination of risk directed therapy in acute myeloid leukemia derived from the MRC AML 10 Trial. *British Journal of Haematology* 1999; **107:** 69–79.
10. Berman E. Chemotherapy in acute myelogenous leukemia: higher dose, higher expectations? *Journal of Clinical Oncology* 1995; **13:** 1–4.
11. Rowe JM & Tallman MS. Intensifying induction therapy in acute myeloid leukemia: has a new standard of care emerged? *Blood* 1997; **90:** 2121–2126.
12. Gonzalez-Llaven J, Rubio-Borja KE & Martinez O. Efficacy of idarubicin plus Ara-C in induction remission of de novo adult acute non-lymphoblastic leukemia. *Haematologica* 1991; **76 (supplement 4):** 128.
13. Reiffers J, Hurteloup P, Stoppa AM et al. A prospective controlled study comparing idarubicin and daunorubicin as induction treatment for acute non-lymphoblastic leukemia in the elderly. *Haematologica* 1988; **73 (supplement 1):** 126.
14. Vogler WR, Velez-Garcia E, Weiner RS et al. A phase III trial comparing idarubicin and daunorubicin in combination with cytarabine in acute myelogenous leukemia: a southeastern Cancer Study Group Study. *Journal of Clinical Oncology* 1992; **10:** 1103–1111.
15. Wiernik PH, Banks PL, Case DC et al. Cytarabine plus idarubicin or daunorubicin as induction and consolidation therapy for previously untreated adult patients with acute myeloid leukemia. *Blood* 1992; **79:** 313–319.
16. Mandelli F, Petti MC, Ardia A et al. A multicentric study from the Italian Co-operative Group GIEMMA. *European Journal of Cancer* 1991; **27:** 750–755.
17. Berman E, Heller G, Santorsa J et al. Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia. *Blood* 1991; **77:** 1666–1674.
18. Arlin Z, Case DC Jr, Moore J et al. Randomized multicenter trial of cytosine arabinoside with mitoxantrone or daunorubicin in previously untreated adult patients with acute nonlymphocytic leukemia (ANLL). Lederle Cooperative Group. *Leukemia* 1990; **4:** 177–183.
19. Wahlin A, Hornsten P, Hedenus M & Malm C. Mitoxantrone and cytarabine versus daunorubicin and cytarabine in previously treated patients with acute myeloid leukemia. *Cancer, Chemotherapy and Pharmacology* 1991; **28:** 480–483.
20. Lowenberg B, Suciu S, Archimbaud E et al. Mitoxantrone versus daunorubicin in induction-consolidation chemotherapy – the value of low-dose cytarabine for maintenance of remission, and an assessment of prognostic factors in acute myeloid leukemia in the elderly: final report of the leukemia cooperative group of the European organization for the research and treatment of cancer and the Dutch-Belgian Hemato-Oncology Cooperative Hovon Group Randomized Phase III Study AML-9. *Journal of Clinical Oncology* 1998; **16:** 872–881.
21. Wheatley K. A systemic collaborative overview of randomised trials comparing idarubicin with daunorubicin (or other anthracyclines) as induction therapy for acute myeloid leukaemia. *British Journal of Haematology* 1998; **103:** 100–109.
22. Burnett AK, Goldstone AH & Milligan DW. Daunorubicin versus mitoxantrone as induction for AML in younger adults given intensive chemotherapy: preliminary results of MRC AML 12 Trial. *British Journal of Haematology* 1999; **105 (supplement 1):** 67(a).
23. Rowe JM, Neuberg D & Friedenberg W. A Phase III study of daunorbicin vs idarubicin vs mitoxantrone for older adult patients (>55 years) with acute myelogenous leukemia (AML). A study of the Eastern Cooperative Oncology Group (E3993). *Blood* 1998; **92:** 1284(a).
24. Bishop JF, Lowenthal PM, Joshua D et al. Etoposide in acute non-lymphoblastic leukemia. *Blood* 1990; **75:** 27–32.
25. Bishop JF, Matthews JP, Young GA et al. A randomized study of high-dose cytarabine in induction in acute myeloid leukemia. *Blood* 1996; **87:** 1710–1717.
26. Weick JK, Kopecky KJ, Appelbaum FR et al. A randomized investigation of high-dose versus standard dose cytosine arabinoside with daunorubicin in patients with previously untreated acute myeloid leukemia: a Southwest Oncology Group Study. *Blood* 1996; **88:** 2841–2851.
*27. Büchner T, Hiddemann W, Wormann B et al. Double induction strategy for acute myeloid leukemia: the effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a randomized trial by the German AML Cooperative Group. *Blood* 1999; **93:** 4116–4124.

28. Rustum YM, Slocum HK, Wang G et al. Relationship between plasma Ara-C and intercellular ARA-CTP pools under conditions of continuous infusion and high-dose ARA-C treatment. *Medical and Pediatric Oncology* 1982; **1:** 33–43.
29. Rustum YM, Riva C & Preisler HD. Pharmacokinetic parameters of 1-β-D-arabinofuranosylcytosine (ara-C) and their relationship to intracellular metabolism of ara-C, toxicity, and response of patients with conventional and high-dose ara-C. *Seminars in Oncology* 1987; **14:** 141–148.
30. White JC, Rathmell JP & Capizzi RL. Membrane transport influences the rate of accumulation of cytosine arabinoside in human leukemia cells. *Journal of Clinical Investigation* 1987; **79:** 380–387.
31. Hiddeman W. Cytosine arabinoside in the treatment of acute myeloid leukemia: the role and place of high-dose regimens. *Annals of Hematology* 1991; **62:** 119–128.
32. Herzig RH, Wolff SN, Lazarus HM et al. High-dose cytosine arabinoside therapy for refractory leukemia. *Blood* 1983; **62:** 361–369.
33. Harousseau JL, Cahn JY, Pignon B et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. *Blood* 1997; **90:** 2978–2986.
34. Cassileth PA, Lynch E, Hines JD et al. Varying intensity of post-remission therapy in acute myeloid leukemia. *Blood* 1992; **79:** 1924–1930.
35. Byrd JC, Dodge RK, Carroll A et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. *Journal of Clinical Oncology* 1999; **17:** 3767–3775.
*36. Grimwade D, Walker H, Oliver F et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML: 10 Trial. *Blood* 1998; **92:** 2322–2333.
37. Sanz MA, Martin G, Rayon C et al. A modified AIDA protocol with anthracycline-based consolidation results in high antileukemia efficacy and reduced toxicity in newly diagnosed PML/RAR α-positive acute promyelocytic leukemia. *Blood* 1999; **94:** 3015–3021.
38. Estey E, Thall P, Andreeff M et al. Use of granulocyte colony-stimulating factor before, during, and after fludarabine pulse cytarabine induction therapy of newly diagnosed acute myelogenous leukemia or myelodysplastic syndromes: comparison with fludarabine plus cytarabine without granulocyte colony-stimulating factor. *Journal of Clinical Oncology* 1994; **12:** 671–678.
39. Lishner M, Curtis JE, Minkin S & McCulloch EA. Interaction between retinoic acid and cytosine arabinoside affecting the blast cells of acute myeloblastic leukemia. *Leukemia* 1989; **3:** 784–788.
40. Hu ZB, Minden MD & McCulloch EA. Direct evidence for the participation of bcl-2 in the regulation of retinoic acid of the Ara-C sensitivity of leukemia stem cells. *Leukemia* 1995; **9:** 1667–1673.
41. Campos L, Rouault JP, Sabido O et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood* 1993; **81:** 3091–3096.
42. Tallman MS, Miller HJ, Zanzig C et al. All-trans retinoic acid, low-dose-cytosine arabinoside and granulocyte colony-stimulating factor in acute myelogenous leukaemia: update of an Illinois Cancer Centre Phase II Pilot Study. *Experimental Hematology* 1996; **24:** 1125. (Abstract).
43. Venditti A, Stasi R, Del Poeta G et al. All-trans retinoic acid and low dose cytosine arabinoside for the treatment of 'poor prognosis' acute myeloid leukemia. *Leukemia* 1995; **9:** 1121–1125.
44. Estey E, Beran M, Pierce S, Kantarjian H & Keating M. All-trans retinoic acid (ATRA) may improve results of chemotherapy in poor prognosis non-APL AML and MDS: a randomised study. *Blood* 1997; **90:** 416a.
45. Estey EH, Thall PF, Pierce S et al. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin +/− all-trans retinoic acid +/− granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. *Blood* 1999; **93:** 2478–2484.
46. Rees JKH & Gray R. Comparison of 1 + 5 DAT and 3 + 10 DAT followed by COAP or MAZE consolidation therapy in the treatment of acute myeloid leukemia: MRC ninth AML trial. *Seminars in Oncology* 1987; **14:** 32–36.
47. Elonen E, Almqvist A, Hanninen A et al. Comparison between four and eight cycles of intensive chemotherapy in adult acute myeloid leukemia: a randomized trial of the Finnish Leukemia Group. *Leukemia* 1998; **12:** 1041–1048.
48. Blaise D, Kuentz M, Fortainer C et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Societe Francaise de Greffe de Moelle. *Journal of Clinical Oncology* 2000; **18:** 537–546.
49. Powles R, Mehta J, Kulkarni S et al. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. *Lancet* 2000; **355:** 1231–1237.
50. Burnett AK, Tansey P, Watkins R et al. Transplantation of unpurged autologous bone marrow in acute myeloid leukemia in first remission. *Lancet* 1984; **2:** 1068–1070.

51. Lowenberg B, Van Den Lelieu J, Goudsmit R et al. autologous bone marrow transplantation in patients with acute myelogenous leukemia in first remission. In Dicke KA, Spitzer G & Jagganath S (eds) *Autologous Bone Marrow Transplantation*, pp 3–7. Houston: University of Texas, 1989.
52. Stewart P, Buckner CD, Bensinger WI et al. Autologous marrow transplantation in patients with acute non-lymphocytic leukemia in first remission. *Experimental Hematology* 1985; **13:** 267–272.
53. Goldstone AH, Anderson CC, Linch DC et al. Autologous bone marrow transplantation following high dose therapy for the treatment of adult patients with acute myeloid leukaemia. *British Journal of Haematology* 1986; **64:** 529–537.
54. Lowenberg B, Verdonk LJ, Dekker AW et al. Autologous bone marrow transplantation in acute myelocytic leukemia in first remission: results of a Dutch prospective study. *Journal of Clinical Oncology* 1990; **8:** 287–294.
55. Gorin NC, Aegerter B, Auvert B et al. Autologous bone marrow transplantation for acute myelocytic leukemia in first remission: a European survey of the role of marrow purging. *Blood* 1990; **75:** 1606–1614.
56. Yeager AM, Kaizer H, Santos GW et al. Autologous bone marrow transplantation in patients with acute non-lymphocytic leukemia, using ex vivo marrow treatment with 4-hydroperoxycyclophosphamide. *New England Journal of Medicine* 1986; **315:** 141–147.
57. Kaizer H, Stuart RK, Brookmeyer R et al. Autologous bone marrow transplantation in acute leukemia: a phase I study of in vitro treatment of marrow with 4-hydroperoxycyclophosphamide to purge tumor cells. *Blood* 1985; **65:** 1504–1510.
58. Chopra R, Goldstone AH, McMillan AK et al. Successful treatment of acute myeloid leukemia beyond first remission with autologous bone marrow transplantation using busulfan/cyclophosphamide and unpurged marrow: the British Autograft Group Experience. *Journal of Clinical Oncology* 1991; **9:** 1840–1847.
59. Gorin NC, Douay L, Laporte JP et al. Autologous bone marrow transplantation using marrow incubated with Asta Z 7557 in adult acute leukemia. *Blood* 1986; **67:** 1367–1376.
60. Gray R & Wheatley K. How to avoid bias when comparing bone marrow transplantation with chemotherapy. *Bone Marrow Transplantation* 1991; **7 (supplement 3):** 9–12.
*61. Burnett AK, Goldstone AH, Stevens R et al. Randomised comparison of addition of autologous bone-marrow transplantation in intensive chemotherapy for acute myeloid leukaemia in first remission: results of MRC AML 10 Trial. *Lancet* 1998; **351:** 700–708.
*62. Cassileth PA, Harrington DP, Appelbaum F et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. *New England Journal of Medicine* 1998; **339:** 1649–1656.
63. Burnett AK, Goldstone AH, Stevens R et al. Allo and auto bone marrow transplant reduce relapse risk in AML in CR1 but do not significantly improve overall survival: results of the MRC AML 10 trial. *British Journal of Haematology* 1996; **93 (supplement 2):** 313.
64. Frassoni F. Commentary and randomised studies in acute myeloid leukaemia: the double truth. *Bone Marrow Transplantation* 2000; **25:** 471–473.
65. Pendry K, Alcorn MJ & Burnett AK. Factors influencing haematological recovery in 53 patients with acute myeloid leukemia in first remission after autologous bone marrow transplantation. *British Journal of Haematology* 1993; **83:** 45–52.
66. Burnett AK, Graham S & Alcorn M. Sustained growth in long term bone marrow culture of AML remission marrow predicts a high probability of remaining relapse free after auto BMT in first complete remission. *Experimental Haematology* 1993; **21:** 1020.
67. Goldstone AH, Perry AR, Robinson LG et al. Stem cell transplants in acute myeloid leukaemia (AML). In Hiddeman W et al. (eds) *Acute Leukaemias VII: Experimental Approaches and Novel Therapies*, pp 906–910, Springer Verlag: Berlin, Heidelberg, New York, 1997.
68. Peterson FB, Lynch MHE, Clift RA et al. Autologous marrow transplantation for patients with acute myeloid leukemia in untreated first relapse or in second complete remission. *Journal of Clinical Oncology* 1993; **11:** 1353–1360.
*69. Slovak ML, Kopecky KJ, Cassileth PA et al. Karyotypic analysis predicts outcome of pre- and post-remission therapy in adult acute myeloid leukemia (AML): A SWOG/ECOG Intergroup Study. *Blood* 1998; **92:** 678a.
*70. Keating S, de Witte T, Suciu S et al. The influence of HLA-matched sibling donor availability on treatment outcome for patients with AML: an analysis of the AML 8A study of the EORTC Leukaemia Cooperative Group and GIMEMA. *British Journal of Haematology* 1998; **102:** 1344–1353.
71. San Miguel JF, Martinez A, Macedo A et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. *Blood* 1997; **90:** 2465–2470.
72. Tobal K, Saunders MJ, Grey MR & Yin JAL. Persistence of RARα-PML fusion mRNA detected by reverse-transcriptase polymerase chain reaction in patients in long-term remission of acute promyelocytic leukaemia. *British Journal of Haematology* 1995; **90:** 615–618.

73. Gallagher RE, Willman CL, Slack JL et al. Association of PML-RARα fusion mRNA type with pretreatment hematologic characteristics but not treatment outcome in acute promyelocytic leukemia: an Intergroup molecular study. *Blood* 1997; **90:** 1656–1663.
74. Yin JAL. Detection of minimal residual disease in acute myeloid leukaemia: methodologies, clinical and biological significance. *British Journal of Haematology* 1999; **106:** 578–590.
75. Grimwade D, Diverio D, Harrison G et al. Detection of minimal residual disease (MRD) in APL by 'real-time' RT-PCR: analysis of cases entered into the UK MRC ATRA trial. *Blood* 1999; **94 (supplement 1):** 625a. (Abstract).
76. Yin JAL, Wheatley K, Rees J et al. Comparison of two chemotherapy regimens, with or without cyclosporin A, in relapsed/refractory acute myeloid leukaemia: results of the UK Medical Research Council. *Blood* 1998; **92:** 231a. (Abstract).
77. Archimbaud E, Thomas X, Leblond V et al. Timed sequential chemotherapy for previously treated patients with acute myeloid leukemia: long-term follow-up of the etoposide, mitoxantrone and cytarabine-86 trial. *Journal of Clinical Oncology* 1994; **13:** 11–18.
78. List AF, Kopecky KJ, Willman CL et al. Benefit of cyclosporin (CsA) modulation of anthracycline resistance in high-risk AML: a Southwest Oncology Group (SWOG) Study. *Blood* 1998; **92:** 312. (Abstract).
79. Gale RP, Horowitz MM, Rees JKH et al. Chemotherapy versus transplants for acute myelogenous leukemia in second remission. *Leukemia* 1996; **10:** 13–19.
80. Sievers EL, Appelbaum FR, Speilberger RT et al. Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 caliceamicin immunoconjugate. *Blood* 1999; **93:** 3678–3684.