International Journal of
HEMATOLOGY
Supplement I

Progress in the Treatment of Acute Myeloid Leukaemia in Adults

Adrian Newland

Department of Haematology, Barts and the London School of Medicine and Dentistry, London, UK

Abstract

There has been important progress in the treatment of Acute Myeloid Leukaemia (AML) in patients under 60 years. A remission rate of 80% can be achieved by several schedules, and 40-45% of patients diagnosed will survive. It may still be possible to improve remission induction treatment eg by intensifying the Ara-C dose although this may only be detectable in an improved disease free survival. The main challenge is to reduce relapse. The risk is pre-determined by a number of powerful risk factors. In the experience of the MRC age, cytogenetics and clearance of blasts from the bone marrow after course 1. Using the later two in combination good risk patients (FAB M3, t(8;21) t(15;17) inv(16)) have a relapse risk of 32%. Poor risk (blasts >15% after course 1 or abnormalities of Chs 5 or 7, 3q- and complex changes have a relapse risk of 82%. All other cases are standard risk and have a relapse risk of 56%. FLT3 mutations have been detected in about 25% of cases and provide additional negative predictive value overall and within each risk group. The assessment of the most effective consolidation treatment must be made taking into account the heterogeneity of the relapse risk. The MRC investigated the role of allo and autoBMT in addition to intensive chemotherapy. The data was analysis on an intent-to-treat or donor vs no donor basis. Although both types of transplant were able to reduce relapse overall and in all risk groups, there was an overall survival advantage only in standard risk patients. Since chemotherapy has improved since this study, there remains uncertainty about the benefit of transplant in all risk groups. Overall this experience has demonstrated that relapse can be reduced with more therapy. It is probable that the limits of conventional chemotherapy have been reached. The new AML15 trial will assess the value of adding the immunoconjugate (Mylotarg) to induction and/or chemotherapy. Improvements in older patients have been less detectable. MRC trials over the last 20 years show an improvement in remission rate (now 65%) but persistent poor survival (12% at 5 years). In the MRC AML11 Trial three induction schedules were compared (DAT vs ADE vs MAC) with DAT being superior. A comparison of a total of 3 vs 6 courses of treatment or the addition of interferon maintenance did not improve results. Newer approaches currently being assessed include resistance modulation; addition of immunoconjugate and minigrafts. New targets for treatment are emerging of which the most interesting is FLT3 inhibitors.

Acute myeloid leukaemia (AML) accounts for less than 1% of human malignancies, although the importance of this disease far outweighs its low incidence. The accessibility of leukaemic tissue has enabled extensive study of the cellular and molecular basis of haematological malignancies and other cancers. So far, however, it has been difficult to transfer this increased understanding into therapeutic benefit.

Some advance has been made in determining which subgroups of patients with AML will do well with conventional therapy, and which will do badly and therefore benefit from alternative, usually experimental, approaches. The morphological classification of AML by the French-American-British (FAB) group [1] has been shown to provide limited prognostic information, with only the M3 variant (acute promyelocytic leukaemia) behaving substantially differently from the other seven subgroups. It has been known for some time that AML evolving from a pre-existing chronic disorder such as myelodysplasia (MDS) or chronic leukaemia has a

poorer outcome [2]. It is now clear that cytogenetic abnormalities of the leukaemic clone, such as translocations of chromosomes 15 and 17 (t15:17), associated exclusively with AML M3, inversions of 16 or t(8:21), confer a good prognosis, although the precise reasons are multi-factorial [3]. Karyotypic abnormalities typically associated with MDS, such as deletions of the long arms of chromosomes 5 or 7, are associated with a poorer outcome [4].

Other cellular features may predict the outcome from chemotherapy. Expression of the multidrug resistance (MDR) protein p-glycoprotein (PGP), which acts as a membrane-associated efflux pump for cytotoxic drugs, correlates inversely with induction of remission and overall survival [5,6]. There is preliminary evidence that other potential mechanisms of resistance to cytotoxic drugs, such as glutathione transferase levels, may be quantified to give prognostic information.

Substantial progress has been made in the treatment of childhood acute lymphoblastic leukaemia, with 75% of children alive and well at 5 years, many of whom will be cured [7] and results in childhood AML have also improved [8]. Conventional chemotherapy for adult AML has been less successful, with less the 30% of patients remaining free of leukaemia in the long term [9]. The poorer results are probably due to inherent differences both in the biology of the disease, and in the ability of the patients to tolerate the intensive treatment required. The majority of deaths are due to leukaemic relapse, often with disease, which is resistant to further chemotherapy; other causes of death are failure of the initial leukaemia to respond to therapy (refractory disease) or death in remission from treatment toxicity. Approximately 10% of deaths can be attributed to each of these remaining causes. Therapeutic advances may therefore be made in three main areas; increasing the proportion of patients entering remission, reducing the rate of relapse once remission has been obtained, and in supportive care.

Single-agent chemotherapy for AML in the 1950s and 1960s led to transient responses in 10-30% of patients. Combination therapy, usually with a combination of an anthracycline plus cytosine arabinoside (ara-C), has become the mainstay of remission-induction treatment. The Medical Research Council study (MRC 10) has been the most successful, with remission rates of around 80% and long-term disease-free survival around 40% [3]. Attempts to improve this led to the MRC 12 study in patients under 60, which looked at the impact of mitoxantrone, the dose of Ara-C and the addition of retinoic acid [10].

The study was undertaken between 1995 and 2002 and three induction treatment questions were addressed. Over 3000 patients with any form of *de novo* or secondary AML, mainly aged up to 59 years, including children, were entered. Initially, patients were allocated between ADE (daunorubicin, Ara-C, etoposide) and MAE (mitoxantrone, Ara-C, etoposide). This randomisation continued until the end of the trial for children. In adults, it was replaced from 1998 by randomisations between standard dose Ara-C (100 mg/m^2 b.d. S-DAT) versus double dose (200 mg/m^2 b.d. H-DAT) and either with or without all-trans retinoic acid (ATRA), both were in conjunction with daunorubicin and thioguanine. Two courses of the allocated induction therapy were administered throughout the trial. Patients with acute promyelocytic leukaemia were not eligible for the ATRA randomisation. The main endpoints were: complete remission rate (CR) and reasons for failure (induction death ID, resistant disease RD), death in CR (DCR), relapse risk (RR), disease-free survival (DFS) and overall survival (OS).

For time-to-event endpoints all percentages are actuarial values, at 5 years for ADE versus MAE and 2 years for the other two randomisations. Results are presented based on follow-up to 1 June 2001, by which time 2947 patients had been entered. Overall outcome (adults/children) in AML12 was: CR=85% (84%/91%), ID=8% (8%/4%), RD=7% (8%/4%), RR=48% (52%/32%), DCR=14% (15%/6%), DFS=45% (41%/64%), OS=44% (40%/64%). The results of the three randomisations are shown in the Table 1.

Table 1.

Endpoint (all figures are %)	Randomisation					
	ADE v. MAE (n=1856)		S-DAT v. H-DAT (n=1010)		ATRA v. not (n=792)	
	ADE	MAE	S-DAT	H-DAT	ATRA	None
CR	86	85	84	84	82	85
ID	7	7	9	9	9	9
RD	7	8	7	7	9	7
DCR	13	15	12	16	19	13
RR	51	43	45	46	53	55
DFS	43	49	48	47	38	39
OS	44	45	50	51	47	45

Table 2.

Endpoint	Randomisation					
	4 v. 5 courses			Chemotherapy v. SCT		
	4	5	OR (CI)	Chemo	SCT	OR (CI)
DCR	6	6	1.31 (0.74-2.33)	6	15	1.42 (0.56-3.57)
RR	42	44	0.95 (0.77-1.16)	70	58	0.68 (0.50-0.94)
DFS	54	53	0.98 (0.81-1.19)	29	36	0.74 (0.55-1.00)
OS	66	62	1.09 (0.87-1.37)	38	42	0.83 (0.59-1.16)

None of the differences between arms is significant, except for RR with ADE versus MAE and DCR with ATRA versus not (both p=0.02). Haematological toxicity with MAE was much greater than with ADE, with both neutrophil and platelet recovery being substantially prolonged after course 2 (p<0.001), with a carry over effect to the first consolidation course as well. The results of AML12 are among the best reported, but attempting to improve outcome further by fine-tuning induction therapy does not appear promising. Longer follow-up of the Ara-C dose and ATRA randomisations is needed, but these preliminary results suggest that the limit to which induction therapy can be further improved with conventional chemotherapy may have been reached.

Regimens containing newer drugs and newer approaches may well be needed to improve remission rates further. Combinations of ara-C with idarubicin, which is not affected by the p-glycoprotein MDR pump, have shown improved remission rates and survival in some studies [11]. A combination of the purine analogue fludarabine with ara-C has also shown promising preliminary results [12] but it is unlikely that these approaches will make major differences.

Maintaining remission, once achieved, probably relies on complete eradication of residual disease. It has been shown that increasing doses of cytotoxic drugs, particularly ara-C, will increase leukaemic cell killing and may increase long-term leukaemia-free survival [13]. However, toxicity is inevitably increased, particularly in older patients, and may abrogate any survival benefit of this approach. The AML 12 study also addressed this point by attempting to optimise consolidation therapy.

All patients entered into AML 12, who were in CR after two course of induction chemotherapy were eligible to receive a course of consolidation chemotherapy followed by randomisation to one or two additional consolidation courses (4 versus 5 courses in total). The additional course was ICE (idarubicin, Ara-C, etoposide) for adults and CLASP (high-dose Ara-C, asparaginase) for children. For standard and poor risk adults (defined as those without favourable cytogenetic abnormalities -t(8;21), t(15;17), inv(16)), a randomisation between chemotherapy versus stem cell transplant (SCT) was also available. If a matched sibling donor was available, the transplant was allogeneic (alloSCT), otherwise it was autologous (autoSCT). The main endpoints were: death in CR (DCR), relapse risk (RR), disease-free survival (DFS) and overall survival (OS) [14].

Results are presented based on follow-up to 1 June 2001, by which time 1076 and 298 patients had been entered into the 4 versus 5 courses and chemotherapy versus SCT randomisations respectively. The results of the randomisations are shown in the table (OR=odds ratio, CI=95% confidence interval, all values are actuarial percentages at 4 years).

RR and DFS were significantly better for SCT compared to chemotherapy, but this did not translate into a significant survival benefit (P=0.3), possibly because of non-significantly more deaths in CR and worse survival from relapse (10% v. 17% at 1 year). None of the differences between 4 versus 5 courses is significant, but CIs are wide and still compatible with some benefit or harm of a fifth course. Within this randomisation, there is no evidence of differences between adults and children or between whether the final course was chemotherapy, alloSCT or autoSCT. However, there was a major interaction (P=0.0001) depending on time period, with a survival benefit for 5 course in the first part of the trial (71% v. 60% at 3 years, P=0.02), and an adverse effect in the second part (60% v. 74%, P= 0.001). Reasons for this interaction will be explored. The results of AML12 are among the best reported (survival from CR in the whole population is 54% at 4 years), but attempting to further intensify consolidation by adding an additional course does not appear to improve outcome, and the same may apply to further intensification of individual course with conventional chemotherapeutic agents.

Many agents induce differentiation in leukaemic blasts *in vitro,* thus raising the prospect of treating leukaemia by overcoming the differentiation block and restoring normal apoptotic mechanisms rather than the traditional approach of cell killing. Leukaemic blasts of the AML M3 subtype (APML) undergo granulocytic differentiation on exposure to the retinoic acid analogue, all-*trans* retinoic acid (ATRA). Susceptibility to induction of differentiation by this agent is due to involvement of the

retinoic acid receptor alpha in the t15 : 17 which characterizes the disease [15]. However, although temporary remissions are obtained, leukaemic relapse is inevitable and cytotoxic chemotherapy is required for eradication of the disease [16]. Differentiation with ATRA does, however, abrogate the severe coagulopathy often associated with this leukaemic subtype, and its use may reduce early deaths. Other differentiating agents, such as vitamin D analogues, have been assessed in AML and MDS with disappointing results but arsenic trioxide, which has been shown to produce partial differentiation in APL cell lines and may also affect the local vasculature by inducing apoptosis in endothelial cells has shown encouraging results with over 90% of patients achieving CR and over 80% demonstrating molecular remission [17].

The metabolic/enzymatic pathways affected by the genetic defects that have lead to the disease are obvious potential targets for treatment. The success of ATRA in Acute Promyelocytic Leukaemia is the early example but the recent results with STI571 (Glivec), the tyrosine kinase inhibitor in Chronic Myeloid Leukaemia give a clear indication of the potential of this approach [18]. An obvious line of approach in AML is the ras-mediated signal transduction pathway, where mutations are seen in up to 50% of cases. Mutations are seen in N-, K-, or H-ras in 10-30% of patients studied. In the up-stream receptor tyrosine kinases, which include Fms, Kit and Flt3, mutations are seen in 30-40% of cases. The most common individual mutation in AML is in Flt3 internal repeats, which occur in 20-25%. This mutation appears to adversely affect prognosis, particularly in respect of relapse, and indications are that it will be particularly useful in defining additional risk in patients in the intermediate cytogenetic risk category [19]. An otherwise, relatively large and heterogenous group with few independent prognostic factors. The mutation is an exciting potential target and a number of novel tyrosine kinase inhibitors are currently under study. Other approaches under investigation include farnesyl transferase inhibitors, which target the post-translational modification of ras to prevent the subcellular localisation necessary for signal transduction.

Cell surface antigens represent the other broad target for novel therapies and these antigens serve as potential targets for both antibody and cellular based therapies. CD33 and CD45 are the most widely studied antigens in AML and have been used as targets for antibody based approaches. Numerous studies targeting the former, utilising gemtuxamab ozogamacin (Mylotarg), are currently underway [20]. There have been a variety of approaches to such treatments including the use of unmodified antibody and antibody-directed radiotherapy and chemotherapy. Cellular-based therapies are much less advanced but studies are underway targeting mutational proteins, such as bcr-abl, and up-regulated proteins, such as WT1.

Allogeneic bone-marrow transplantation (BMT), where normal marrow from an HLA-matched donor is used, has the advantages of rescue with marrow known to be free of disease, and a putative graft vs. leukaemia effect, which may be curative [21]. It has the disadvantages of substantial toxicity and the lack of availability of a suitable donor for up to 80% patients. Procedure-related mortality may be as high as 40%, or higher if the donor is unrelated rather than a matched sibling; deaths are mainly due to graft vs. host disease or opportunistic infection. However, relapse is rare and long-term disease-free survival may be achieved in about 40-60% of patients [22].

Improvements in understanding of the HLA system have improved tissue typing for BMT but, ironically, have made it more difficult to find perfectly matched donors. Extensive efforts have been made to increase the unrelated marrow donor registry data base to improve donor availability.

Autologous bone marrow transplantation (ABMT) is more widely available, as the patient's own bone marrow is used, having been removed and frozen in remission prior to high-dose chemotherapy. Procedure-related mortality is less than 10%, although there is a documented risk of transplanting leukaemia back into the patient [23], and there is little evidence of any graft vs. leukaemia effect. As a result, leukaemia-free survival (40-50%) is less than for allogeneic BMT [24]. Several large randomized studies have been conducted to show that ABMT confers benefit over conventional chemotherapy [22]; however, results to date are conflicting, and the MRC continue to address this question. Autologous transplantation may also be performed using progenitor cells derived from peripheral blood (peripheral blood stem-cell transplant, PBSCT). More rapid haemopoietic reconstitution using PBSCs may reduce the toxicity and cost of the procedure, but it remains to be seen whether there is any survival benefit.

Evidence for a potentially curative graft vs. leukaemia effect in allogeneic BMT recipients has rekindled interest in agents, which activate the anti-tumour properties of the immune system. Immunomodulatory agents may improve long-term leukaemia-free survival by increasing eradication of minimal residual disease which persists after high-dose chemotherapy. Promising results were obtained with interleukin 2 in pilot studies [25], but the large randomized studies which are required to confirm the efficacy of this agent have not been done. Linomide, a synthetic compound, is a potent activator of T lymphocytes and natural killer cells *in vivo*, and may reproduce a syndrome similar to graft vs. host disease, however, a randomized trial of its efficacy after ABMT for AML failed to confirm this. Granulocyte-macrophage colony-stimulating factor (GM-CSF) will increase monocyte anti-tumour activity, although no clinical trials have yet been performed [26].

An alternative approach has been to utilise a lower dose, non-myeloablative, preparative regimen designed not to eradicate the malignancy, but to provide sufficient immunosuppression to achieve engraftment of an allogeneic stem cell graft, thus allowing the development of an immune graft versus malignancy effect. Such an approach allows the use of allotransplantation for older

patients and those with co-morbidities that preclude high-dose chemoradiotherapy. Such a strategy is attractive and early results are encouraging, however further trials in a controlled clinical setting are required [27].

Mechanisms whereby leukaemic cells become resistant to cytotoxic drugs are obvious targets for therapeutic intervention. Cyclosporin A and verapamil will inhibit the MDR protein pump PGP [28], although verapamil is required in concentrations which preclude clinical use, and cyclosporin, although used with some success, is at the limit of its tolerability at doses which inhibit PGP. Newer analogues of these agents with more selective action on PGP, such as PSC 833 and dexaverapamil, are being evaluated but their clinical benefit appears to be limited in the long term probably reflecting other, as yet poorly identified, mechanisms of leukaemic cell resistance.

Recombinant growth factors, such as GM-CSF and granulocyte colony stimulating factor (G-CSF) have had a significant impact on the morbidity of cancer chemotherapy in general. Fears that these agents may stimulate leukaemic growth have largely been allayed by randomized trials. A reduction in the duration of neutropenia after autologous BMT, and possibly after conventional chemotherapy as well, reduces infectious morbidity [29]. A significant impact on morbidity from deep-seated fungal infection, such as pulmonary aspergillosis, is anticipated from the use of these agents and preliminary data in this respect are encouraging. Unfortunately, studies have failed to show that G-CSF or GM-CSF alter the mortality of elderly patients undergoing chemotherapy for AML [30,31]. Growth factors are used extensively for the mobilization of peripheral blood stem cells prior to collection, and have proved invaluable in this respect [32]. G-CSF and GM-CSF have also been used to stimulate AML blasts into mitosis and thus 'sensitize' them to the effects of cycle-specific cytotoxic agents. Despite exciting *in vitro* data, the results of clinical trials have been disappointing [33]. The impact of growth factors is thus far confined mainly to improvements in supportive care.

Intensive chemotherapy, with or without haemopoietic stem-cell support, remains the mainstay of treatment for AML and is likely to do so for some considerable time. Only a minority of patients can expect to be cured. New developments in our understanding of the cellular and molecular biology of AML are occurring with great rapidity and the products of these are already entering clinical trials. It is only through this understanding that major advances in the treatment of AML are likely to be made.

References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of acute leukaemia. *Br J Haemotal.* 1976;33:451.
2. Mertelsmann R, Thaler HT, Gee TS, et al. Morphological classification, response to therapy and survival in 263 adult patients with acute non-lymphoblastic leukaemia. *Blood.* 1980;56:773-81.
3. AK Burnett, AH Goldstone, RMF Stevens, et al. Randomised comparison of addition of autologous bone marrow transplantation to intensive chemotherapy for acute myeloid leukaemia in first remission: AML 10 trial. *Lancet.* 1998;351:700-708.
4. Fenaux P, Morel P, Rose C, Lai JL, Jouet JP, Bauters F. Prognostic factors in adult de novo myelodysplastic syndromes treated by intensive chemotherapy. *Br J Haematol.* 1991;77: 497-510.
5. Wood P, Burgess R, MacGregor A, Yiu Lin JA. P glycoprotein expression on acute myeloid leukaemic blast cells predicts response to chemotherapy and survival. *Br J Haematol.* 1994;87:509-514.
6. Zochbauer S, Gsur A, Brunner R, Kyrle PA, Lechner K, Pirker R. P-glycoprotein expression as unfavourable prognostic factor in acute myeloid leukaemia. *Leukaemia.* 1994;8:974-977.
7. Chessels JM, Bailey C, Richards SM, for the MRC Working Party on Childhood Leukaemia. Intensification of treatment and survival in all children with lymphoblastic leukaemia: results of MRC trial UKALL X. *Lancet.* 1995; 345:143-148.
8. LC Riley, IM Hann, K Wheatley, et al. Treatment-related deaths during induction and first remission of acute myeloid leukaemia in children treated on the Tenth Medical Research Council Acute Myeloid Leukaemia Trial (MRC AML10). *Br J Haematol.* 1999;106:436-444.
9. Preisler HD, Anderson K, Rai J, et al. The frequency of long term remission in patients with acute myeloid leukaemia treated with conventional maintenance chemotherapy: a study of 760 patients with a minimum follow up of six years. *Br J Haematol.* 1989;71:189-194.
10. Burnett AK, Wheatley K, Goldstone AH, et al. Attempts to improve induction treatment in AML patients under 60 years: The impact of mitoxantrone; ARA-C dose and retinoid acid: Results of MRC AML12. *Haematol J.* 2002;3(Suppl 1):159.
11. Vogler WR, Volaz-Gurcia E, Weiner RS, et al. A phase III trial comparing idarubicin and daunorubicin in combination with cytarabine in acute myelogenous leukaemia: a Southeastern Cancer Study Group study. *J Clin Oncol.* 1992;10:1103-1111.
12. Visani G, Tosi P, Zinzani PL, et al. FLAG (fludarabine plus high dose cytarabine + G-CSF): an effective and tolerable protocol for the treatment of poor risk myeloid leukaemias. *Leukemia.* 1994;8:1842-1846.
13. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with AML. *N Engl J Med.* 1994;331:896-903.
14. K Wheatley, AK Burnett, B Gibsox, et al. Optimising consolidation therapy: Four versus five courses SCT versus chemotherapy - Preliminary results of MRC AML12. *Haematol J.* 2002;3(Suppl 1):159.
15. Grignani F, Fagioli M, Alcalay M, et al. Acute promyelocytic leukemia: from genetics to treatment. *Blood.* 1994;83:10-25.
16. Fenaux P, Le Deleyu MC, Castaigne S, et al. Effect of all-trans retinoic acid in newly diagnosed acute promyelocytic leukaemia. Results of a multicentre randomised trial. *Blood.* 1993;11:3241-3249.
17. Soignet SL, Frankel SR, Douer D, et al. United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia. *J Clin Oncol.* 2001;19:3852-3860.
18. Druker BJ, Talpaz M, Resta DJ, et al. Efficacy and Safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med.* 2001;344:1031-1037
19. D Kottaridis, RE Gale ME Frew, et al. The Presence of a FLT3 Internal Tandem Duplication in patients with Acute Myeloid Leukemia (AML) adds important prognostic information to Cytogenetic Risk Group and Response to the First Cycle of Chemotherapy: Analysis of 854 Patients from the United Kingdom Medical Research Council AML 10 and 12

Trials. *Blood.* 2001;98:1752-1759.
20. Russo D, De Angelo D, Castaigne S, et al. Report of the Safety and efficacy of gemtuxumab ozogamicin (Mylotarg) given in combination with cytarabine and daunorubicin in patients with acute myeloid leukemia: Phase 1. *Haematol J.* 2002;3(Suppl 1):159.
21. Horowitz MM, Gale RP, Sondel PM. Graft versus leukaemia reactions after bone marrow transplantation. *Blood.* 1990;65: 555-562.
22. Zittoun RA, Mandelli F, Willemze R, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukaemia. *N Engl J Med.* 1995;332:217-223.
23. Brenner MK, Rill DR, Holladay MS, et al. Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients. *Lancet.* 1993;342:1134-1137.
24. McMillan AK, Goldstone AH, Linch DC, et al. High dose chemotherapy and autologous bone marrow transplantation in acute myeloid leukaemia. *Blood.* 1990;76:480-488.
25. Lim SH, Newland AC, Kelsey SM, et al. Continuous intravenous infusion of high dose recombinant interleukin 2 for acute myeloid leukaemia a phase II study. *Cancer Immunol Immunother.* 1992;34:337-342.
26. Williams MA, Kelsey SM, Collins PW, Gutteridge CN, Newland AC. Administration of GM-CSF activates monocyte reactive oxygen species secretion and adhesion molecule expression in vivo in patients following high dose chemotherapy. *Br J Haematol.* 1995;90:31-40.
27. Chajaraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using nonmyeloablative conditioning regimen *Blood.* 2002;99:1071-1078.
28. Jiang XR, Macey MG, Collins PW, Newland AC. Characterisation and modulation of drug transport kinetics in K562 c16 daunorubicin-resistent cell line. *Br J Haematol.* 1994;86: 547-554.
29. Gisselbrecht C, Prentice HG, Bacigalupo A, et al. Placebo controlled phase III trial of lenograstim in bone marrow transplantation. *Lancet.* 1994;343:696-699.
30. Stone RM, Berg DT, George SL, et al. GM-CSF after initial chemotherapy for elderly patients with acute myeloid leukaemia. *N Engl J Med.* 1995;332:1678-1683.
31. Dombret H, Chastang C, Fenaux P, et al. A controlled study of rhG-CSF in elderly patients after treatment for AML. *N Engl J Med.* 1995;332:1678-1683.
32. Juttner CA, To LB. Peripheral blood stem cells for therapeutic use. In: Burnnett A, Armitage J, Newland AC, Keating A, eds. *Haematological Oncology* 3. Cambridge, Cambridge University Press:147-168.
33. Estey E, Thall PF, Kantarjian H, *et al.* Treatment of newly diagnosed acute myelogenous leukaemia with GM-CSF before and during continuous infusion with high dose ara-C and daunorubicin: comparison to patients treated without GM-CSF. *Blood.* 1992;79:2246-2255.