Review article

A review of the current status and techniques of allogeneic bone marrow transplantation for treatment of leukaemia

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SUMMARY Bone marrow transplantation is now an accepted component in the overall therapy of acute and chronic (myeloid) leukaemia for some selected patients. Some of the obstacles to success have been partially overcome.

Many advances in supportive care have been made. *Pneumocystis carinii* and herpes simplex infections are preventable. Effective decontamination of the gastrointestinal tract for bacteria and fungi is now readily achievable and may have reduced the risk of serious systemic infections. New antibiotics which, in combination, are effective in life-threatening infections are under study.

Recent developments in the prevention or amelioration of graft versus host disease (GvHD) have included T lymphocyte depletion in the donor marrow and the use of the fungal polypeptide cyclosporin A. Less than 10% of patients would now be expected to succumb to this complication.

Outstanding problems remaining to be resolved are the improvement in the antileukaemic conditioning prior to transplantation and the prevention or treatment of cytomegalovirus infection in the seropositive recipient. This infection can cause pneumonitis and is currently the single most frequent transplant related cause of mortality.

That bone marrow transplantation could protect against supralethal irradiation was first demonstrated by Lorenz¹ in 1951 in mice. This work had followed the original observation by Jacobson² of protection by splenic shielding. For the next two decades attempts at marrow transplantation in man were frustrated mainly by a lack of knowledge of transplantation immunology, with failure to engraft or fatal graft versus host disease.

The first successful marrow transplant in man was reported by Robert Good³ and coworkers in 1968. This was for a child with severe combined immune deficiency, who remains well to this day. In the ensuing 15 years much progress has been made, and over 3000 transplants have now been performed mainly

as part of the management of patients with leukaemia, the subject of this review.

Indications for bone marrow transplantation in leukaemia

With the exception of a small minority of children with acute lymphoblastic leukaemia (ALL) who relapse *off* treatment, all patients with relapsed leukaemia irrespective of subsequent remission and those with refractory leukaemia ultimately die of their disease. Bone marrow transplantation provides the only possibility of cure in this setting with the chance of cure ranging from 13%⁴ (relapsed/refractory disease) to possibly 50% for ALL in second remission in children. With limited resources⁵ we must unfortunately select patients

Table 1 *Indications for bone marrow transplantation (where major histocompatibility complex matched sibling available)*

Acute lymphoblastic leukaemia	(a) First remission
	Age <14 yr } WBC >100 × 10 ⁹ /l (all subtypes)
	Age ≥14 and <45 yr } B-ALL
	Age ≥14 and <45 yr } Chromosomal translocation
	(b) Second remission—all types
Acute myeloblastic leukaemia	Age <45 yr
	First remission, second remission or early relapse
Chronic granulocytic leukaemia	Age <45 yr
	First chronic phase or possibly accelerated phase
	Age <45 yr

most likely to benefit.

Children with acute lymphoblastic leukaemia (ALL), including the thymic (T-ALL) variant but not B-cell ALL, can currently expect a cure rate with available chemotherapy of up to 75%.⁶ Subgroups with a poor prognosis, in whom bone marrow transplantation may increase the chance of cure, may be identifiable but with improvements in the results of chemotherapy the prognostic distinctions are becoming less clear cut and most bone marrow transplantation groups would now recommend transplantation in second remission (see Table 1). Adults with the same disease fare less well for unknown reasons.^{7,8}

The long term cure rate in acute myeloblastic leukaemia (AML) has remained at around 10–20% and unless current intensive therapies improve this result,⁹ this disease will remain a target for bone marrow transplantation until more selective anti-leukaemia treatment is discovered.

Chronic granulocytic leukaemia is a less common disease but is uniformly fatal since eradication has proved to be unattainable. The existence of a matched donor should be considered an absolute indication for bone marrow transplantation in a young adult. One important question is the timing of the procedure, although this should probably be during the first chronic phase.

Although many other haematological malignancies have been treated by, or are theoretically amenable to bone marrow transplantation, the indications are as yet unclear.

Donor selection

Because of the high risk of severe graft versus host disease (GvHD) after incompatible grafts, the majority of bone marrow transplantations are performed between siblings who have inherited the same major histocompatibility complex genes from both parents and are thus HLA A, B, C, D and DR identical. The D locus is tested in a mixed lymphocyte reaction but this may, in future, be supplemented by a test employing epidermal cells (MLECR)¹⁰ and even tests to detect potential graft

versus leukaemia reactivity.¹¹ Preliminary data suggest that some HLA antigens are associated with a reduced risk of developing GvHD—for example, B8 and B15 whereas B18 appears to increase this risk.¹²

The use of additional tests to ensure maximum compatibility would have the unwanted effect of reducing the pool of potential donors which because there is only a 1 in 4 chance of any one sibling being major histocompatibility complex identical, is already limited, whereas the recent work of Powles and colleagues¹³ with mismatched but haplotype-identical family donors suggests, that at least in children, complete major histocompatibility complex identity is not always necessary. Insufficient data are as yet available to recommend the use of HLA identical unrelated donors. Occasional patients have an identical twin who should usually be the donor of choice especially in the older patient so as to avoid fatal graft versus host reaction, but in younger recipients who are less susceptible to GvHD an HLA identical sibling may be a superior donor with the possibility of an advantageous graft versus leukaemia reaction.

The donor should be fit to undergo a general anaesthetic including heparin anticoagulation and be free from transmissible disease.

Antileukaemia/immunosuppressive conditioning

Most bone marrow transplantation groups have, with minor modifications, used the regimen developed by the Seattle BMT team of high dose cyclophosphamide (120 mg/kg) followed by total body irradiation (TBI). Haemorrhagic cystitis due to the acrolein metabolite of cyclophosphamide can be largely prevented by the concurrent administration of Mesna¹⁴ or by forced alkaline diuresis. There is considerable debate on the optimum technique for administration of TBI, the various possibilities are: (i) low dose rate (<0.05 Gy/min) to give ≈ 10 Gy TBI; (ii) fast dose rate up to 0.25 or 0.5 Gy/min with a reduced total dose—that is, 7.5 Gy or 5.0 Gy; (iii) fractionated TBI with higher total doses administered—that is, up to 17.5 Gy. In theory

slower dose rates or fractionated TBI will be less toxic to normal tissues especially the lung but *may* be less antileukaemic. Fast dose rates are more toxic to normal tissues, but lung toxicity appears to be a problem only where the total received dose exceeds 8 Gy.¹⁵ This question is not resolved and relevant data are presented in the Results section.

Although more effective anti-leukaemic protocols have been devised such as that from the UCLA group known as SCARI,¹⁶ the antileukaemic benefit was offset by increased toxicity. Encouraging antileukaemic conditioning has been reported from the Johns Hopkins Group without TBI. Using high dose busulphan (16 mg/kg) combined with cyclophosphamide their relapse rate is very low, although pneumonitis remains a problem.¹⁷

For bone marrow transplantation in children the Westminster group have devised a new programme employing high dose steroid, cytosine arabinoside, VM26, daunorubicin and total body irradiation with encouraging preliminary results (AJ Barrett, personal communication 1983).

Marrow harvest, in vitro treatment and infusion

Under general or spinal anaesthesia, and usually with heparin (100 IU/kg preservative free) anticoagulation, sufficient marrow is aspirated from the pelvis to yield at least 2×10^8 nucleated cells/kg of recipient weight. The aspirated volume ranges from 500 to 1000 ml for adults and consists mainly of peripheral blood. The whole procedure rarely takes more than one hour. No more than 5 ml should perhaps be aspirated at any one point to reduce blood contamination. Some form of filtration to remove large particles or bony fragments should be employed. It is a good plan if donors, especially females, have one unit of blood taken and stored for 2–3 wk before marrow donation at which time the blood is reinfused and many of the hazards of blood transfusion are avoided. Some discomfort is usual for 2–3 days but donors are discharged from hospital within 24 h.

Most groups infuse whole marrow immediately after the completion of TBI. Marrow stem cells have the ability to “home” into the marrow cavity. In ABO incompatible donor recipient pairs—for example, A or B into O, we now recommend depletion of the red cells from the marrow suspension in preference to isoagglutinin reduction in the recipient by plasma exchange. This is readily achieved with either sedimentation or more completely using Ficoll-Metrizoate in a cell washer.¹⁸

Several groups are now exploring the possible benefit of in vitro T lymphocyte depletion for prevention of acute GvHD (see GvHD). Ficoll

Metrizoate isolation of the mononuclear fraction free of plasma and red blood cells allows the use of antibodies with complement for this purpose.¹⁹

After marrow infusion “take” can usually be seen on marrow sampling by 10 days in most recipients, and the circulating white cell count reaches $1 \times 10^9/l$ by about 3 wk.

Prevention of graft versus host disease

Graft rejection is rare in bone marrow transplantation for acute leukaemia because of the profound immunosuppression induced by the disease, its treatment and the antileukaemic pretransplant conditioning. On the other hand GvHD was until recently the single most frequent cause of failure of bone marrow transplantation. This is more common in adults than in children and is fatal in about 25% of severe cases. The most commonly used prophylaxis is a three month programme of methotrexate originally developed in Seattle from experiments in dogs.²⁰ Despite methotrexate between 50 and 70% of recipients develop acute GvHD, although with improved patient health at the time of bone marrow transplantation (better selection) the incidence and severity are declining. Acute GvHD may be seen from as early as seven days to as late as 100 days after bone marrow transplantation. The main targets are the skin with epidermal loss, the gastrointestinal tract with severe diarrhoea and mucosal loss and the liver where the small bile ducts are selectively destroyed. This complication appears to be triggered by infection—for example, cytomegalovirus, in many recipients, is less common in patients who are nursed in laminar airflow with effective microbial decontamination,²¹ and is quite uncommon in experiments with germ-free animals.

The novel fungal polypeptide cyclosporin A which has a selective inhibitory effect on T cell function was discovered by Borel²² and first used in bone marrow transplantation by Powles.^{23,24} Cyclosporin A does not reduce the incidence of GvHD but does reduce the severity. With its use the incidence of fatal GvHD is reduced to less than 8%. The major disadvantage of cyclosporin A is nephrotoxicity²⁵ which requires careful monitoring of renal function and cyclosporin A blood levels. Cyclosporin A is usually given for six months or more. A small number of patients develop GvHD on withdrawal of the drug.

In 1968 van Bekkum and coworkers demonstrated that acute GvHD was dependent upon the infusion of competent lymphocytes with the marrow, and was prevented by their removal in vitro.²⁶ Subsequent studies in several sub-human

species have implicated T lymphocytes specifically and have shown that by T cell depletion bone marrow transplantation becomes possible across major histocompatibility complex barriers.^{27–29} The Munich bone marrow transplantation group have obtained promising results in man by incubating donor marrow with conventional (extensively absorbed) rabbit antihuman T sera.³⁰ More recently the Royal Free Bone Marrow Transplantation Group have explored the use of the monoclonal (murine) anti-T antibody OKT3 (Ortho Pharmaceuticals, Raritan, USA). In vitro opsonisation with intravenous infusion of the treated marrow was followed by a 24% incidence of severe (grade II+) GvHD.³¹ Three of 25 cases were fatal in the context of coincidental cytomegalovirus infections in two, and with D locus incompatibility in the third. Subsequently 10 consecutive bone marrow transplantation recipients received marrow partially depleted of T lymphocytes (mean 71%) by in vitro treatment of marrow with OKT3 and rabbit complement. Three developed grade II or greater GvHD which was fatal in one (10%). Current studies with total T cell depletion in vitro suggest that GvHD can also be prevented in man, at least between major histocompatibility complex matched pairs.³²

Another method of T cell depletion in vitro has been that devised by Reisner and colleagues who, using a time-consuming method of lectin separation and sheep red blood cell rosetting, have achieved a T cell depleted fraction which has not led to the development of GvHD, but second marrow infusions have frequently been required to ensure engraftment.³³

Treatment of established acute GvHD has not been entirely successful with the use of cyclosporin A²³ or (conventional) antithymocyte globulin (ATG), the best results are seen with intravenous high dose bolus methyl prednisolone.³⁴

Chronic GvHD may occur in up to 25% of bone marrow transplantation recipients and is manifest by skin lesions (pigmentation/depigmentation and telangiectasia), by lichen planus-like lesions in the mouth and by an "obstructive" small bile duct lesion in some. The skin lesion may progress to disabling scleroderma frequently accompanied by the sicca syndrome and other autoimmune phenomena. Careful treatment with modest doses of steroids and azathioprine are usually successful although it is likely that the patients will remain permanently immunodeficient.

Support care for bone marrow transplantation recipients

With the recent improvements in prevention of

GvHD the major residual problem in bone marrow transplantation is death due to infection.³⁵ With the routine use of reverse barrier isolation exogenously acquired organisms are uncommon but disease caused by reactivation of, or invasion by, pathogens carried by the host is not prevented.

Attempts are made to decontaminate the bone marrow transplantation recipient (see Table 2 for details) with non-absorbable antibiotics and antifungal agents taken by mouth. Antiseptics are applied to the nose, skin and vagina. Only sterile food and drinks are allowed. Cotrimoxazole is given as prophylaxis for pneumocystis and some bacteria³⁶ and, increasingly, acyclovir to prevent reactivation of herpes simplex and zoster.^{37,38} But acyclovir does not prevent reactivation (or acquisition) of cytomegalovirus. A variable number of patients suffer a cytomegalovirus infection which, if it progresses to pneumonitis may be fatal in up to 90% of cases, and this is becoming one of the greatest obstacles to successful bone marrow transplantation. Prophylactic cytomegalovirus immunoglobulin has been used in both Seattle and UCLA with some success to prevent cytomegalovirus pneumonitis in seronegative recipients.^{39,40} Antifungal prophylaxis is still a major problem since the only effective therapeutic agent available to date is intravenous amphotericin B, which is potentially toxic. Orally administered ketoconazole alone proved ineffective in bone marrow transplantation recipients, in a recent study, probably because of its reduced absorption due to the effect of TBI on the GI tract.^{41–43} We currently recommend a combination of high dose oral (suspension) amphotericin or nystatin with ketoconazole twice daily. But ketoconazole should not be used concurrently with cyclosporin A since displacement from protein binding sites can lead to inappropriately high levels of cyclosporin A and toxicity. Air filtration should prevent the acquisition of aspergillosis.

Most patients have little appetite and in some cases there is a degree of malabsorption which requires intravenous feeding including vitamin supplements for 2–4 wk. Although no controlled trials are available this approach prevents metabolic complications such as hypocalcaemia/hypomagnesaemia and probably reduces the incidence of infection, accelerates marrow regeneration and reduces both morbidity and mortality due to GvHD and infection.

Platelets (to keep the platelet count $\geq 20 \times 10^9/l$) and red blood cells are best given as concentrates. Few units now use prophylactic granulocytes. Their use has been ineffective, costly and potentially dangerous—for example, cytomegalovirus transfer.

An important development in administration of intravenous preparations and in blood sampling has

Table 2 Suggested prophylactic antimicrobial care for bone marrow transplantation recipients

Agent		Antimicrobial activity	Period of use
Ketoconazole* + Amphotericin B suspension	oral	Fungi	– 10 days → + 6 months
Cotrimoxazole	oral	<i>Pneumocystis carinii</i>	– 9 days → + 6 months
Neomycin + Colistin	oral	Bacterial GI contamination	– 9 days → WBC $\geq 1 \times 10^9/l$
Acyclovir	oral/IV	Herpes simplex and zoster	0 → ? 6 months
Penicillin	oral	Pneumococci	for many years

*Not with cyclosporin A.

been the widespread adoption of the right atrial Hickman catheter.⁴⁴ This silastic tube has a Dacron cuff which lies in a skin tunnel on the chest wall and provides it with self retaining properties. The (heparinised) catheter can be “capped off” and allows the patient full mobility. Unfortunately the use of such catheters has been followed by an increase in bacteraemias due to *Staphylococcus epidermidis* and diphtheroids. Although these organisms do not appear particularly pathogenic, the use of empirical broad spectrum antibiotics may increase the risk of invasive fungal infection.

In the first three weeks after bone marrow transplantation the main pathogens are herpes simplex, Gram-positive cocci and Gram-negative bacteria. Around two to three weeks there may be fungal infections—mainly due to *Candida albicans*. An empirical programme for the urgent management of any fever in these patients should include an aminoglycoside and a penicillin—for example, mezlocillin, ticarcillin, or a cephalosporin. Failure to respond after three to four days should be followed by the introduction of intravenous amphotericin B. From three to four weeks the granulocyte count is rapidly returning to normal. Although bacterial and fungal infection are still seen the major pathogen is cytomegalovirus for which no effective treatment is as yet available. From three months onwards many patients develop herpes zoster. Pneumococcal infection is being increasingly recognised especially in patients with chronic GvHD who may manifest the haematological features of hyposplenism and fail to make appropriate antibodies perhaps because of excessive suppressor activity.⁴⁵ Penicillin prophylaxis with or without vaccination should be given in this situation.

Patients with uncomplicated grafts do not recover full immunity for up to two years and those with chronic GvHD may remain immunocompromised for life.

Results

The results of all series are beset by a number of

variables and uncertainties which render all conclusions rather tentative. The critical influence of age is indicated by the fact that in children and adolescents the results are better than those in adults, where it is better to be in the twenties than in the thirties and in whom the chance of success is small in the forties. Then again the follow-up for most cases is still relatively short and the incidence of late complications such as autoimmune disease, and second neoplasms cannot be predicted.

Meanwhile secular trends operating through ever increasing experience, precision and better care for patients make non-synchronous comparisons hazardous and the decreased incidence of GvHD is a notable instance where better protective isolation and anti-infection prophylaxis may have been critical.

One interesting association is that patients surviving moderate or severe GvHD have a lower leukaemic relapse rate than those without, the highest relapse rate being where the donor is an identical twin. This is usually interpreted as being an effect of the graft action on the residual leukaemic cells and although that is quite possibly correct other explanations are tenable and no method has yet been found to regulate the degree of GvHD to an optimum.

A lot of attention has been paid to the form of total body irradiation, in particular the dose rate and fractionation, and in Seattle comparative trials of different fractionation schedules have been made. In one synchronous randomised trial of AML in first remission those receiving fractionated radiation fared better than those receiving a single dose of radiation, but less well than a previous series who had a single fraction, while in other series no conclusive differences have emerged. The optimum schedule has yet to be identified and the data which follow are given without reference to the irradiation schedule.

ACUTE LEUKAEMIAS IN FIRST REMISSION

Acute lymphoblastic leukaemia

No large series of bone marrow transplantations for

treatment of acute lymphoblastic leukaemia in first remission have yet been reported, although five such patients are included in a report from the Westminster Transplant team.⁵¹ All remain well in remission from 220–1126 days. The difficulty in this condition, where chemotherapy is constantly improving, is to determine which individual patients really need a transplant.

Acute myeloblastic leukaemia

With the poor results of conventional chemotherapy most bone marrow transplantation centres have in recent years considered patients with acute myeloblastic leukaemia in first complete remission suitable for bone marrow transplantation. The results are encouraging and surprisingly uniform. Approximately 50% of patients appear to be cured of their disease regardless of the institution.

The results from the two centres with the largest experience are detailed:

The Seattle bone marrow transplantation team initially published details of 19 patients in 1979.⁵² Three subsequent patients were conditioned with the same protocol (cyclophosphamide 60 mg/kg $\times$ 2 and 9.2 Gy TBI). Of the 22, 12 are in continued complete remission. The median disease free survival will be ≥ 44 months.

More recently similar patients have been entered into a randomised study to determine the role of fractionated TBI versus 10 Gy at one exposure.⁵⁴

In total 75 patients with acute myeloblastic leukaemia in first remission have been transplanted and followed for 1 to 5 yr. Only six have relapsed (5 within 14 months). Thirty-nine patients are surviving and with the possible exception of the Boston series⁹ these results are clearly superior to any obtained, to date, with conventional chemotherapy.⁵³

Similar good results have been reported by the Royal Marsden bone marrow transplantation team.⁵⁵ Fourteen of 22 patients who received cyclophosphamide 120 mg/kg and 10 Gy TBI were surviving in remission compared with a matched control population receiving chemoimmunotherapy of whom only eight of 29 were surviving in complete remission. The same group have recently reported their results for allogeneic transplantation in a variety of patients using mismatched donors¹³ with encouraging results for children, but poor results in adults. Some of the benefit of allogeneic bone marrow transplantation appears to be due to a graft versus leukaemia effect, which cannot, as yet, be dissected from the GvHD phenomenon. In patients with GvHD the leukaemia relapse rate is two-fifths of that in patients without GvHD.⁵⁶ It is hoped that a reduction in GvHD will not be accompanied by a

loss of the graft versus leukaemia effect. The ultimate fate of these patients is unknown but since in the earliest Seattle cases of bone marrow transplantation after relapse those who survived two years did not relapse thereafter, it may be hoped that most are cured.

Patients transplanted in second or subsequent remission

In acute myeloblastic leukaemia actuarial analysis of the Seattle cases suggests a relapse risk of 50%. Seven of 24 patients were alive and disease-free at median 2 years.⁴⁹ In ALL, 4 of 12 patients transplanted following fractionated TBI (14 Gy) are alive and disease free 657–991 days after bone marrow transplantation—which is not significantly better than 22 receiving single fraction (10 Gy) TBI of whom six are alive and disease free at 4 yr or more.⁵⁰

Acute leukaemia in relapse

The only large published series of patients treated uniformly (cyclophosphamide and TBI) is from Seattle. Of 74 patients 11 are surviving, nine leading entirely normal lives from 4 to 8 yr after bone marrow transplantation. The predicted relapse rate (censoring for transplant-related deaths) was 64%.⁴⁶ More intensive conditioning programmes such as “SCARI” from the UCLA team resulted in a lower relapse rate but equivalent mortality because of an increase in toxicity.¹⁶

The Seattle group have recently reported their initial results using different schedules of fractionated irradiation in ALL.⁴⁷ The total received dose ranged from 1200 rads to 1750 rads over two to seven days in addition to cyclophosphamide at 120 mg/kg. Three patients received additional dimethylmyleran. Ten of 41 died of transplant related complications with no evidence of leukaemic recurrence. One died on day 19 with persisting leukaemia and 27 of the remaining 30 have had a leukaemic relapse. Nine relapses were extramedullary. The three remaining patients were in remission for 523–770 days (as of 1 June 1981).

A study in relapsed AML of 200 rads daily $\times$ 6 plus cyclophosphamide in 23 patients has also been reported.⁴⁸ Nine patients died of transplant related complications, eight relapsed but six (25%) are alive and disease free from 756–1306 days after bone marrow transplantation (as of 1 June 1981).

It should be emphasised that most deaths after bone marrow transplantation in relapsed leukaemia are attributable to non-leukaemic causes due to the poor condition of patients at the time of bone marrow transplantation.

Chronic granulocytic leukaemia

Data from 13 centres were recently summarised by

Champlin *et al.*⁵⁷ Sixteen patients in blast crisis transplanted with identical twin marrow have an actuarial relapse risk of 63% and disease free survival at 30 months of 19%. Only 5 of 45 receiving an allogeneic bone marrow transplantation whilst in blast crisis survived one year with a disease free survival of 8%, whereas 13 of 17 patients with identical twin bone marrow transplantation during the first chronic phase remain disease free from 2 to 6 years later. Of 62 who have received an allogeneic bone marrow transplantation during the first chronic phase only one has relapsed and the disease free survival at 2 years is 75%. For bone marrow transplantation in the accelerated phase this falls to 35%.

With the likely long latent period to re-emergence of the Philadelphia chromosome and its clinical expression we must await the final outcome, but with reason for optimism.

Conclusion

Allogeneic bone marrow transplantation is presently the most effective method of rescuing patients with acute leukaemia from lethal/curative chemoradiotherapy, although we await with interest the current experiments in autologous bone marrow transplantation. For patients with poor prognosis disease—for example, acute myeloblastic leukaemia more than half are currently being cured with improved methods of preventing the potentially lethal complications of GvHD and infection. It is not unrealistic to anticipate an 80% cure rate in the near future. If this does happen more patient groups could be included and further exploration of the use of (family) HLA mismatched donors or matched unrelated donors would widen further the population of patients who might benefit. Some caution must, though, be expressed about the long term immunological reconstitution of a high proportion of bone marrow transplantation recipients.

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