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# TRANSPLANTATION OF HEMATOPOIETIC PROGENITOR CELLS WITH EMPHASIS ON THE RESULTS IN CHILDREN*

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**SUMMARY: Thomas ED. (Fred Hutchinson Cancer Research Center, Seattle, Washington, USA). Transplantation of hematopoietic progenitor cells with emphasis on the results in children. Turk J Pediatr 1995; 37: 31-43.**

**Attempted human allogeneic marrow transplants in the 1950's and 60's were largely unsuccessful. In the past two decades the probability of success has improved steadily depending on the type and stage of disease. All marrow transplant teams have observed that the results for children are better than for adults. Long-term survival and apparent cure rates range from about 90 percent for non-malignant diseases transplanted early to 15 percent for patients with advanced leukemia. Most marrow transplants have involved an HL-A matched sibling donor but, more recently, through the worldwide marrow donor registries a matched unrelated volunteer marrow donor can be found for many patients without a family donor. Current research involves new preparative regimens for elimination of malignant cells, better prevention of graft-versus-host disease (GVHD), and the use of hematopoietic growth factors and cytokines. Autologous transplants, which use the patient's own marrow, are increasing. The hematopoietic stem cell, which is responsible for marrow regeneration after a transplant, has been isolated and characterized. Stem cells for transplantation can now be obtained from the peripheral blood after mobilization of these cells by chemotherapy or hematopoietic growth factors.** *Key words: bone marrow transplantation, hematopoietic progenitor cells, children.*

In 1949, Leon Jacobson and his colleagues, while studying the mechanism of death from total body irradiation (TBI), showed that shielding the spleen allowed mice to recover from otherwise lethal irradiation[1]. Lorenz et al.[2] then showed that infusion of syngeneic marrow also provided an irradiation protection effect. In 1955, Main and Prehn showed that mice protected with an allogeneic marrow infusion would accept permanently a skin graft from the marrow donor[3]. Ford, et al. showed by cytogenetic techniques that the irradiation protection effect was due to the transfer and survival of hematopoietic cells from the donor[4]. In 1956, Barnes and Loutit treated leukemic mice by irradiation and marrow transplantation[5]. These studies formed the cornerstones of the field of marrow transplantation.

The potential therapeutic application of supralethal chemo-irradiation and marrow grafting to leukemia and other diseases of the marrow was soon investigated by several groups. Dr. Joseph Ferrebee and I and our colleagues in

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Cooperstown, N. Y., began a human marrow grafting program in 1955. Our first publication in 1957 discussed our reasons for concluding that allogeneic marrow grafting in our species would be very difficult[6]. We were able to show that large quantities of marrow could be infused intravenously with safety, but only one patient showed transient engraftment. Subsequently, two children with refractory acute lymphoid leukemia who had an identical twin were given supralethal irradiation and a syngeneic marrow infusion[7]. Their prompt hematologic recovery and well-being provided proof that an intravenous infusion of marrow could protect against otherwise lethal irradiation although their leukemia recurred in a few months.

We began in 1955 to study the dog as a readily available outbred animal commonly used for transplantation research and amenable to clinical procedures comparable to those used for human patients[8]. We found that dogs given 1000 R (9.2 Gy) and an infusion of their own stored marrow routinely survived and lived a normal life span[9], while only about 10 percent of dogs given an allogeneic marrow infusion survived[10]. It was clear from studies in both rodent models and dog models that a successful allogeneic marrow graft depended upon close histocompatibility matching between donor and recipient[11]. Under the impetus of kidney grafting, human tissue typing based on human leukocyte antigens (HL-A) proceeded rapidly in several laboratories. We developed dog antisera for detection of DL-A antigens which enabled us to show that marrow grafts between littermates matched with these sera were almost always successful if followed by a few weeks of immunosuppression with methotrexate to suppress the graft-versus-host reaction[12].

My colleagues and I began in 1967 to assemble and train the Seattle team of doctors and nurses for critical care of patients with leukemia undergoing allogeneic marrow grafts who would be without marrow function and subject to opportunistic infections. In November of 1968 Dr. Robert Good and his colleagues at the University of Minnesota carried out the first marrow transplant from a matched sibling for an infant with an immunological deficiency disease[13]. In March of 1969, we carried out our first transplant for a man in the blastic phase of chronic myeloid leukemia who had a matched sister to serve as donor. The experimental background and the early clinical successes and problems were reviewed in the New England Journal of Medicine in 1975[14]. Thus, the "modern" era of human marrow transplantation began.

At the present time marrow grafting is being applied to a variety of malignant, non-malignant, and genetically determined diseases. The tremendous increase in application of allogeneic marrow grafting around the world, numbering more than 10.000 per year, has been documented by the International Bone Marrow Transplant Registry[15].

## Types of Marrow Transplants

A marrow transplant is really a transplantation of primitive hematopoietic progenitor cells (PHPCs) that have the capacity to proliferate and repopulate the marrow spaces. These PHPCs can be obtained from marrow, peripheral blood or cord blood. The type of the transplant is determined by the relationship between the donor and the recipient.

*Syngeneic Transplant*: A transplant between identical twins.

*Autologous Transplant*: A transplant using the patient's own PHPCs that have been collected before intensive therapy and returned after the therapy.

*Allogeneic Transplant*: A transplant between donor and recipient of different genetic origin.

*Matched Sibling Transplant*: A transplant between donor and recipient siblings who have inherited the same two human leukocyte antigen (HL-A) regions on chromosome 6, one from each parent (a genotypic match).

*Matched Unrelated Donor Transplant*: A transplant using a donor who is unrelated to the patient but phenotypically matched for the HL-A antigens.

During the 1970's and most of the 1980's the majority of marrow transplants utilized matched sibling donors. Therefore, the longest and most extensive data involve transplants of this type which will be described below. Only about one-fourth of patients will have an HL-A identical sibling. Examination of the extended family may identify members who share one HL-A haplotype, with the other haplotype being phenotypically matched or partially mismatched[16]. These donors can be used with success comparable to that of matched siblings. The U.S. National Marrow Donor Program now has registered more than one million volunteer donors and similar panels are being enlarged in several cooperating countries. Transplants using unrelated donors who are phenotypically matched or differing by only one HL-A antigen are showing promising results[17].

If a suitable allogeneic marrow donor cannot be found, the patient's own marrow my be removed, stored and given back after the preparative regimen. The pirinciples of long-term cryopreservation of marrow and autologous marrow transplantation are well established[18]. Autologous marrow transplant regimens and procedures for removing malignant cells that might be contaminating the marrow are being studied by many marrow transplant teams[19,20]. Long term survivals are being reported. Autologous transplants avoid the problems associated with GVHD but lose the advantage associated with the graft-versus-leukemia effect[21]. The reported results show a higher incidence of recurrent malignancy after autologous grafts compared to allogeneic grafts but, in many instances, almost equivalent long-term survival due to the absence of problems associated with GVHD.

It has long been known that hematopoietic progenitor cells are present in the circulating blood and that these cells can repopulate the marrow after lethal irradiation[22]. It has now been shown that the number of these stem cells in the blood is greatly increased after sublethal chemotherapy or after the administration of hematopoietic growth factors such as granulocyte colony stimulating factor (G-CSF). With continuous-flow centrifuges, quantities of stem cells suitable for transplantation can be collected from the blood, thus avoiding the need of the operating room and a general anesthesia for marrow procurement[23]. The major use of peripheral blood stem cells has been for autologous grafting, either alone or combined with marrow. Allogeneic grafts can now be achieved with peripheral blood stem cells, and this technology may well replace marrow as the source of stem cells.

## Problems with Marrow Grafting

*Graft-Versus-Host Disease*: GVHD is a major problem even when donor and recipient are HL-A identical siblings. Several immunosuppressive drugs have been evaluated to suppress the graft-versus-host reaction. The best regimen yet reported is the combination of a short course of methotrexate combined with six months of cyclosporine[24]. Although removal of T-cells from the donor marrow in vitro is effective in preventing GVHD, it is associated with an increased risk of graft failure and/or recurrence of malignancy[25]. Chronic GVHD occurs in approximately one-third of recipients. Treatment with alternate day glucocorticoids over several months will control most, but not all, cases[26].

*Relapse*: Recurrence of disease following the marrow graft is the major cause of failure of transplants for malignancy. The initial regimen in Seattle consisted of high dose cyclophosphamide (CY) and 10 to 12 Gy total body irradiation (TBI). Since then, many marrow transplant teams, in an effort to achieve a greater kill of malignant cells, have employed a variety of high dose chemotherapy regimens with or without TBI. Santos and Tutschka developed a regimen of cyclophosphamide combined with busulfan without irradiation. The regimen is equivalent to the cyclophosphamide-total body irradiation regimen but avoids the exposure to irradiation and is easier to use[27].

Efforts to increase the intensity of the transplant preparative regimen are limited by life-threatening damage to organs other than the marrow, especially the lung, liver and heart. This problem is illustrated by a Seattle study of leukemic patients prepared with two doses of CY and randomized to receive either 12 GY or 15.75 Gy of TBI[28]. Although the higher dose of irradiation resulted in fewer leukemic relapses, there were more deaths from organ toxicity, so that disease-free survival was not improved.

As conventional chemotherapy of malignant disease before transplantation and the marrow transplant regimens have become more intensive, veno-occlusive disease (VOD) of the liver is a more frequently recognized problem and an increasing cause of death[29]. Treatment is largely palliative. Although the kidney does not seem to be a primary target of the marrow grafting regimens, renal failure is not uncommon because of nephrotoxic drugs, associated liver failure, and fluid and electrolyte problems.

Following an allogeneic marrow graft and especially with immunosuppressive treatment of GVHD, patients are profoundly immunoincompetent and at great risk for opportunistic infections[30]. Bacterial disease, P.carinii and some fungal diseases can be controlled with appropriate antibiotics, at times supplemented with granulocyte transfusions. Herpes simplex and Varicella zoster can now be prevented with acyclovir. A leading cause of death was cytomegalovirus (CMV) pneumonia. The use of CMV negative blood products (if donor and recipient are sero-negative) and the treatment or prevention of CMV infection with ganciclovir have resulted in a reduction in CMV-related problems[31,32].

## Results of Marrow Grafting in Children

Marrow transplant teams have uniformly reported that children show better long-term disease-free survival than adults, perhaps due to a lower incidence and severity of GVHD. In the 1970's most transplants were carried out in patients with far advanced disease. In the past decade, transplants have been carried out much earlier in the course of the disease. However, even now many children are referred because the referring physician believes that they have one or more poor prognostic factors, which makes comparison to conventional therapy difficult. Based on expected outcome as well as a few randomized prospective trials, it is clear that marrow grafting is a preferred therapy for many diseases and stages of disease, and, in many instances, the only curative approach.

## Allogeneic Marrow Grafts

*Acute Lymphoblastic Leukemia (ALL)*: Fig. 1 shows a Kaplan-Meier plot of the disease-free survival and Fig. 2 the probability of relapse for children transplanted from a matched sibling in Seattle from 1972 to 1992, analyzed in August, 1994. It should be kept in mind that the earlier patients did not have the benefit of recent improvements in technology and that most patients transplanted in first remission were chosen on the basis of poor prognostic factors.
The International Bone Marrow Transplant Registry (IBMTR) reported the experience with children transplanted from matched siblings in 1984-1990, showing a disease-free survival probability of 0.62 for those in first remission, 0.42 for second remission and 0.18 for relapse[33].

*Acute Myeloid Leukemia (AML)*: In 1979 the Seattle team reported a series of patients with AML transplanted in first remission from a matched sibling donor after preparation with CY and TBI[34]. Of the six children in that report, four are living in remission 15 to 17 years later. Fig. 3 shows the survival of all children with AML transplanted in Seattle from 1972 to 1992 and analyzed in July, 1994.

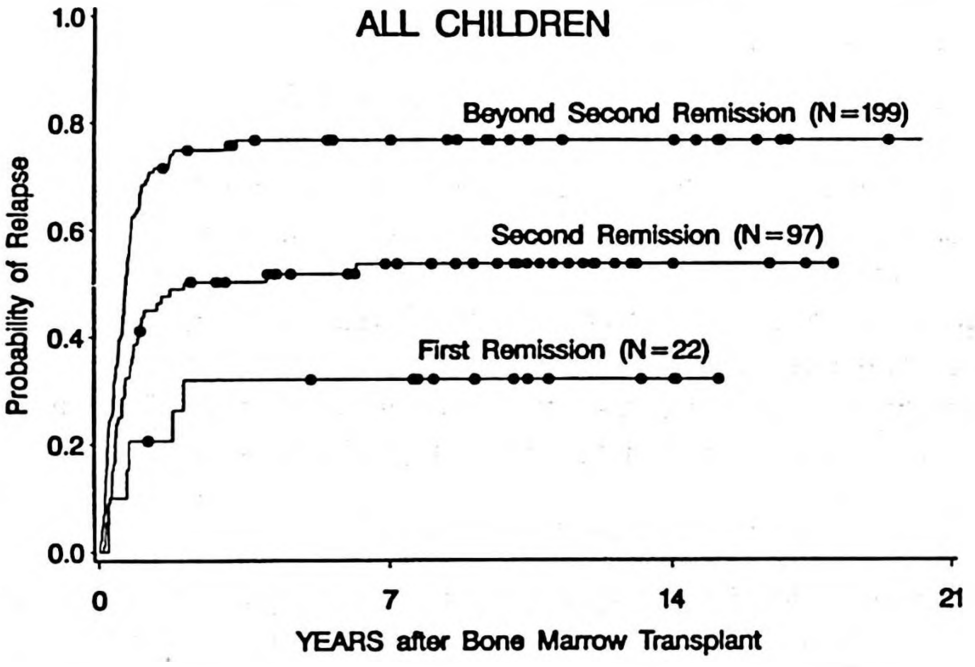


Fig. 1: Kaplan-Meier product limit estimates for the probability of disease-free survival for all patients with ALL under the age of 18 years transplanted in Seattle from a matched sibling donor between 1972 and 1992 and analyzed in August, 1994.

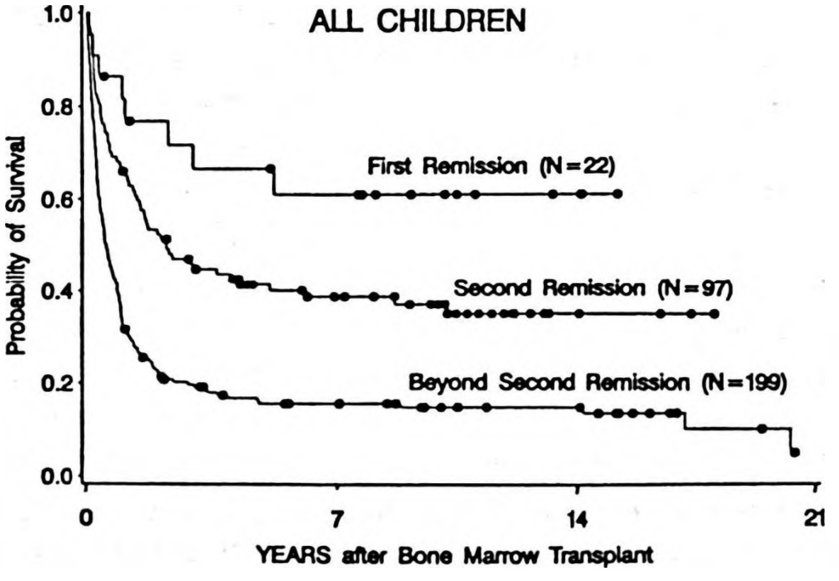


Fig. 2: Kaplan-Meier product limit estimates for the probability of relapse for all patients with ALL under the age of 18 years.

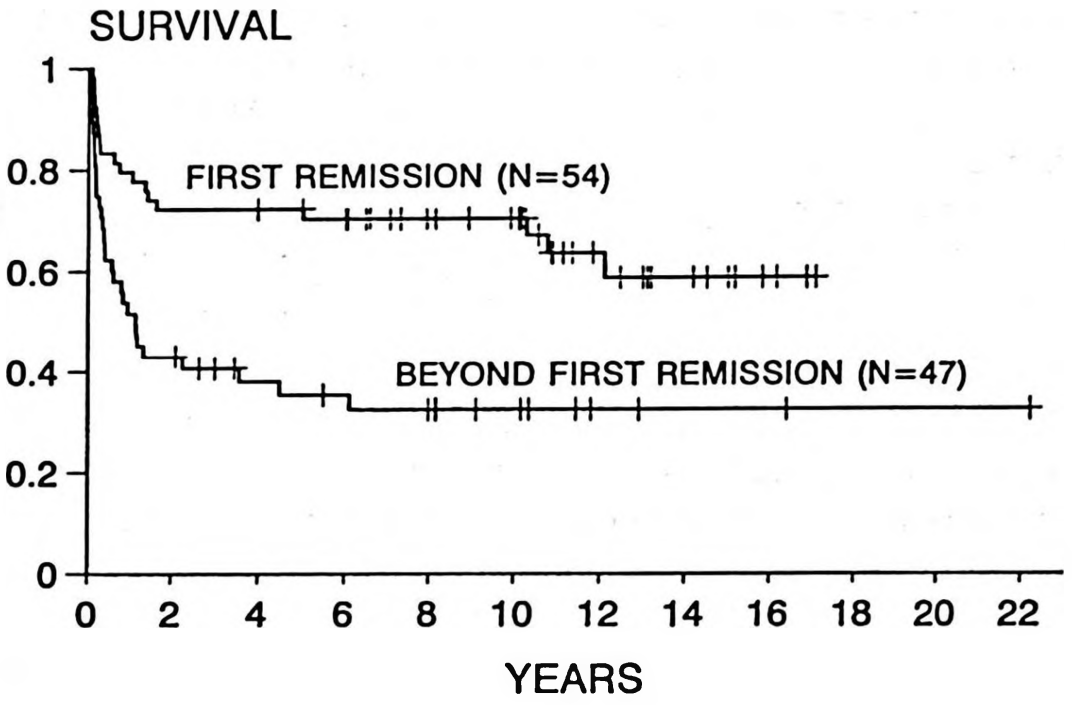


Fig. 3: Kaplan-Meier product limit estimates for the probability of disease-free survival for all patients with AML under the age of 18 years transplanted in Seattle from a matched sibling donor between 1972 and 1992 and analyzed in July, 1994.

The Children's Cancer Group reported a study of 508 patients with AML. Those achieving a remission with a matched sibling were allocated to marrow transplantation after preparation with CY-TBI, while those without a donor received additional combination chemotherapy. With all patients followed for at least eight years, the survival was 47 percent for those treated with marrow transplantation compared to 41 percent for those treated with combination chemotherapy[35].

In a subsequent study, the Children's Cancer Group described a 55 percent three-year survival in a series of 16 such children transplanted after preparation with BU-CY[36]. In Seattle 18 children who achieved a first remission have been prepared with BU-CY and given a matched sibling marrow graft (Sanders et al., unpublished observations). One died early. Two relapsed and are again in remission after a second transplant. With follow-up to six years, the survival probability is 0.94, and the disease-free survival probability is 0.83.

The IBMTR reported a large series of children transplanted with a variety of regimens. They found a seven-year disease-free survival probability of 0.62 for children transplanted in first remission, 0.38 for those transplanted in second remission and 0.21 for those transplanted in relapse[33].

*Chronic Myeloid Leukemia (CML)*: Philadelphia chromosome positive CML is an uncommon disease in children. The Seattle team has recently published a prospective study in 142 patients with CML ages 7-60 randomized to receive either CY-TBI or BU-CY[27].

Only seven of those patients were under the age of 20. Two have relapsed, both had received BU-CY and are clinically well on interferon, a survival probability of 1.0 and disease-free survival of 0.71. The IBMTR reported a probability of 0.60 at seven years for these children transplanted from a matched sibling donor while in chronic phase[33]. All reports show a poor long-term survival for transplantation in accelerated or blastic phase. Since this disease is not cured by chemotherapy, marrow transplantation should be considered early after diagnosis if a matched donor can be found.

*Juvenile Chronic Myelogenous Leukemia*: Few reports of marrow transplantation for this very rare but aggressive disease are available. The Seattle team reported 14 such patients given matched or partially matched marrow after preparation with CY-TBI[37]. Six patients became long-term survivors, showing that this disease can be cured with a marrow grafting regimen.

*Hodgkin's Disease and Non-Hodgkin's Lymphomas*: The variety of these diseases and their rarity has made it difficult to acquire definitive data on the results of marrow grafting as compared to conventional therapy. In general the results parallel the leukemias with good survival if the transplant is carried out early in the course of the disease and very poor results for those transplanted in the end stages.

*Aplastic Anemia*: The Seattle team has employed CY (50 mg/kg on each of four days) as the preparative regimen for patients with severe aplastic anemia given a marrow graft from a matched sibling donor. With methotrexate (MTX) given post-grafting to prevent GVHD, the long-term survival was about 60%. With the combination of MTX with cyclosporine (CSP) to prevent GVHD survival was improved. Most recently, the addition of antithymocyte globulin (ATG) to the regimen has produced long-term survival of better than 90% for both children and adults[38]. Sanders et al. have provided details of the Seattle experience with children transplanted for severe aplastic anemia prior to the regimen with ATG[39].

*Thalassemia Major*: The first patient to be cured of Thalassemia major was prepared with dimethyl busulfan and CY and transplanted from a matched sibling in Seattle 13 years ago[40]. The patient is well and growth and development have been normal. The largest series and the best results of transplantation for Thalassemia major have been reported by Laucarelli et al. using a preparative regimen of BU-CY[41]. They described an approximate 90 percent long-term disease-free survival for patients who are transplanted before complications arise, and approximately 65 percent for patients with iron overload or hepatitis. This disease is but one of some 40 genetically determined diseases of the marrow that have been treated with marrow transplantation.

## Autologous Marrow Transplantation

In the past five to seven years, the number of autologous marrow transplants being performed for a variety of diseases has been increasing rapidly. Because of the relatively shot follow-up time, few definitive reports of disease-free survival in children are available.

Billett et al. reported 51 children with ALL in relapse after a long first remission who were prepared with combination chemotherapy and TBI and given an autologous marrow graft purged with monoclonal antibodies. The event-free survival at three years was 0.53[42]. Schmid et al. described 22 children with advanced ALL after treatment with the German BFM regimens who were prepared with VP-16 and TBI and given an autologous marrow graft. The probability of event-free survival at three years was 0.18[43]. Lonnerholm et al. reported 25 children, two in first remission with poor prognostic indicators and 23 in second or third remission, given an autologous graft of purged marrow. After a median follow-up of 50 months, the probability of disease-free survival was 0.65[44]. Colleselli et al. reported 56 children with ALL in second or subsequent remission given an autologous marrow graft with a four-year event-free survival of 0.21[45]. The Children's Cancer Group described 58 children with AML in first remission given an autologous marrow graft after preparation with BU-CY[36]. The actuarial disease-free survival at three years was 0.51.

A few reports describe studies of high-dose therapy and autologous marrow transplantation in children with lymphoma[46-49]. These reports show that long-term disease free survival in some children can be achieved, but controlled trials have not been done.

From these reports it is evident that autologous marrow grafting after intensive preparative regimens is effective in achieving disease-free survival in patients with acute leukemia. Much longer follow-up time is needed for full evaluation.

## Syngeneic Marrow Transplantation

A special form of "autologous" stem cells for transplantation is a syngeneic marrow transplant. The normal twin donor cells are not contaminated with malignant cells, a major source of concern with the usual autologous transplant. Syngeneic marrow transplants can lead to long-term survival in patients with acute leukemia transplanted in first remission[50] or in relapse[51] and with CML[52]. Survival after a syngeneic transplant is comparable to that of a matched sibling transplant because the higher relapse rate associated with a syngeneic transplant is offset by the absence of complications due to GVHD.

## Growth and Development

Growth and development are of major importance and concern for children who have had a major illness and who undergo intensive therapy and a marrow

graft. Chronic GVHD and its treatment are further threats to normal growth and development. Long-term studies of these problems are ongoing in many marrow transplant centers. In brief, growth and development appear near normal in those patients treated for non-malignant disease who are prepared for grafting with CY and/or other immunosuppressive regimens. Those children who receive intensive cytotoxic therapy and/or irradiation show a spectrum of long-term effects ranging from normal to severe impairment. Details of these studies have been provided by Sanders[53].

## Recent Developments

The use of growth factors anad cytokines produced by recombinant molecular biological techniques is among the most exciting recent developments in marrow transplantation. In clinical trials it has been shown that G-CSF and granulocyte-macrophage colony stimulating factor (GM-CSF) can accelerate recovery of granulocytes after both allogeneic and autologous marrow grafts with consequent significant shortening of the time in the hospital[54]. Several other growth factors and combinations of growth factors are in clinical trial. The recent cloning of thrombopoietin offers hope for facilitating recovery of platelets after marrow grafting[55-57]. Biological response modifiers including IL-2[58], cloned T-cells[59] and interferon[60] are being explored for a possible increased effect against malignant cells. Of great interest is the fact that infusions of donor T-cells that have not been exposed to IL-2 can bring about remission of relapsed chronic myeloid leukemia after marrow grafting[61].

Identification of the "stem cell" responsible for repopulation of marrow has long been a goal of experimental hematologists. Progress has been made in its isolation[62]. Monoclonal antibodies that recognize stem cells now may be used in conjunction with cell separation technology for separation and concentration of stem cells or for selective removal of malignant cells for autologous marrow transplantation[63]. Perhaps it will soon be possible with the "right" combination of growth factors to culture and expand purified stem cells in vitro.

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