

## Non-myeloablative conditioning for hemopoietic stem cell transplantation - does it work?

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### Abstract

Allogeneic bone marrow or peripheral blood stem cell transplantation traditionally uses myeloablative regimen for conditioning to enable grafting of donor's stem cells. Animal experiments have shown that a milder non-myeloablative conditioning regimen does allow engraftment to occur. Non-myeloablative conditioning regimens are low-intensity immunosuppressive treatment given to the recipient before infusion of donor's stem cells. It was reported to have decreased immediate procedural mortality, in particular those secondary to acute graft versus host reaction. However, it did give rise to higher risks of graft rejection, tumour tolerance and disease progression. Fortunately, appropriately administered donor lymphocyte infusion has been shown to establish full donor chimerism (complete donor stem cell grafting in the recipient's bone marrow) and potentiate anti-tumour effect (graft versus tumour reaction). The reduction of immediate transplant mortality allows the procedure to be carried out in older age groups, patients with concomitant diseases that otherwise would have made the patients unfit for the procedure, patients with non-malignant disorders such as congenital immune deficiencies, autoimmune disorders or thalassaemia majors. The regimen also allows transplantation of genetically manipulated haemopoietic stem cells (gene therapy) to be carried out more readily in the immediate future. Lastly, the regimen may serve as a platform for immunotherapy using specific T cell clones for anti-tumour therapy with or without the knowledge of known tumour antigen.

*Key words:* Stem cell, transplantation, immunotherapy.

### INTRODUCTION

Bone marrow transplantation (BMT) was introduced in the early 1960s by the pioneering work of individuals like Mathe, McFarland and Donnall Thomas. The development and history of hematopoietic stem cell transplantation has been recently reviewed by E. Donnall Thomas,<sup>1</sup> who had been conferred a Nobel Award for his contribution to this field. The history of hemopoietic stem cell transplantation has illustrated the development of a new treatment through the close interaction between advances in laboratory science and advances in clinical science.

Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly being used to treat a variety of hematological malignancies,<sup>2</sup> and has an expanding role in the management of non-malignant disorders. Conventionally, stem cell infusion is preceded by the administration of a combination of high dose chemo-radiotherapy. This myeloablative conditioning regimen is aim

to eliminate the patient's own bone marrow and immune system, thereby creating a microenvironment suitable for marrow engraftment and preventing graft rejection by the host. A second aim in appropriate patients is to eliminate any residual malignant disease. The transplant procedure is considered as a rescue procedure following a myeloablative treatment. The exact type of conditioning depends on the disease being treated. Most conditioning regimens consist of radiotherapy combined with alkylating agents, etoposide and cytarabine. However, by comparing numerous protocols used for over 20,000 transplants reported by the international BMT registry, no difference or clear advantage could be documented for different conditioning regimens.<sup>3</sup> Attempts to improve the disease-free survival by increasing the intensity of the conditioning regimen, thereby eradicating host-derived stem cells more effectively, have resulted in unacceptable toxicity.

The use of allogeneic BMT is limited by the toxicity of the high dose chemo-radiotherapy conditioning regimen, hence decreasing the overall effectiveness of allografting and precluding its use in elderly patients or those with concurrent medical disorders.

### ORIGIN OF NON-MYELOABLATIVE CONDITIONING

There is considerable evidence that the curative potential of allogeneic HSCT is mediated, in part at least, by an immune-mediated graft-versus-leukemia (GVL) effect. The most conclusive evidence of the GVL effect is the reinduction of complete remission by the infusion of donor lymphocytes in patients who had relapsed after allogeneic BMT.<sup>4</sup> The importance of this immune reaction between donor-derived immunocompetent T lymphocytes and host-type tumor cells has also accounted for the significantly better anti-leukemia effects induced by allogeneic BMT compared with autologous BMT and transplants between identical twins.<sup>5,6</sup> The increased relapse rate in recipients of T-cell-depleted allografts and the inverse correlation noted between relapse and severity of graft-versus-host disease (GVHD),<sup>7</sup> further support the importance of GVL effect in allogeneic HSCT. This indicates that the main therapeutic component of allogeneic HSCT may be ascribed to T-cell-mediated GVL effects rather than a physical elimination of all tumor cells by high doses of chemo-radiotherapy given as part of the conditioning before transplantation. It suggests that myeloablative therapy may not be a prerequisite of stem cell engraftment. Hence, the main role of the transplant procedure may be the induction of a state of host-versus-graft tolerance by a non-myeloablative regimen and giving donor derived T-lymphocytes the opportunity to recognize and eradicate host-derived tumor cells and abnormal stem cells.

### WHAT IS NON-MYELOABLATIVE CONDITIONING?

The main aim of using a non-myeloablative conditioning regimen is to maximize transient immunosuppression in order to facilitate stem cell engraftment rather than to eradicate the tumor cells and hence reduce the toxicity of allografting. The subsequent mixed donor chimerism can then be used as a platform for the effective delivery of an anti-leukemia effect through donor lymphocyte infusion. Although induction of GVL can be initially accomplished

by T-lymphocyte-enriched donor derived stem cells, due to concomitant administration of cyclosporin as mandatory anti-GVHD prophylaxis, some of the GVL effect may be suppressed. Therefore, immunocompetent T lymphocytes obtained from the donor can be added in graded increments while controlling for disappearance of tumour / host cells on one hand and signs of GVHD on the other. It is hoped that this approach can deliver an equivalent or superior anti-leukemic effect to that observed after a conventional allograft with a significant reduction in transplant-related mortality.

### ANIMAL EVIDENCE

The establishment of donor hemopoiesis after allogeneic HSCT depends on 2 critical steps, the initial homing or lodgment of transplanted hemopoietic progenitors and their subsequent proliferation in the bone marrow microenvironment. By understanding the biology of stem cell engraftment, a model of hemopoietic progenitors homing has recently been developed.<sup>8</sup> Following this, the concept of myeloablation to open space/ niches as a prerequisite for marrow stem cell engraftment has been challenged by studies showing high rates of engraftment in non-myeloablated mice.

Brecher *et al.*<sup>9</sup> using Y chromosome analysis on mice congenic for subtypes of phosphoglycerate kinase, shows engraftment of normal marrow into normal hosts without myeloablation, within 2 to 13 weeks posttransplantation with percent engraftment ranging from 16% to 25%. Saxe *et al.*<sup>10</sup> using similar approaches, have found 0% to 16% engraftment in marrow or spleen at 4 to 6 weeks or 6 months post transplantation. In 1994, Steward *et al.*<sup>11</sup> have demonstrated the early engraftment and long term chimerism after transplantation of a donor marrow to normal nonirradiated host mice. Storb *et al.*<sup>12</sup> have shown that a sublethal dose of total body irradiation, 200 cGy, in combination with post-transplant cyclosporine and mycophenolate mofetil allows the generation of stable mixed chimerism with minimal toxicity.

In syngeneic model studies, the primary determinant of engraftment appears to be the donor:host stem cell ratio. If an adequate stem cell numbers are infused, a mixed chimerism state is possible to be achieved by using non-myeloablative therapy. Stewart F *et al.*<sup>11</sup> have demonstrated the data supporting the above hypothesis using low intensity conditioning

regimen which is not myelotoxic. This observation has had a major impact on the development of clinical protocols with a minimally myeloablative conditional regimen but maximizing the stem cell inoculum.

### CLINICAL EXPERIMENTS AND RESULTS

Based on the animal evidence, a number of clinical experiments using non-myeloablative conditioning regimens have been reported since early 1998. A selected list is shown in Table 1.

Fludarabine based regimen has been widely used in the early studies. It is a highly immunosuppressive purine analogue. Using either fludarabine or 2-chlorodeoxyadenosine, in combination with cytosine arabinoside and idarubicin, the MD Anderson group in Houston<sup>13</sup> reported engraftment of allogeneic peripheral blood stem cell harvested from HLA-identical siblings in patients with advanced leukemia or myelodysplasia. 8 out of 14 patients achieved complete remissions that lasted a median of 60 days post transplantation. 6 of 8 patients achieving remission showed more than 90% donor cells between 14 to 30 days post infusion and 3 of 4 patients remaining in remission between 60 and 90 days continued to have more than 80% donor cells. In a subsequent paper, the same group used fludarabine in combination with cyclophosphamide or cisplatin and cytosine arabinoside in patients with lymphoid malignancies.<sup>14</sup> Donor engraftment was seen in 11 of 15 patients. The remaining 4 patients had prompt recovery of their autologous hematopoiesis. 8 of the engrafted patient achieved complete remission but follow up (median of 180 days) was too short to assess the long term anti-tumor effect. Only one treatment-related death occurred in a patient who had active hepatitis C pre-transplantation. The same group also explored using fludarabine or cladribine with melphalan as a reduced-intensity preparative regimen<sup>15</sup> in 86 patients who had a variety of hematological malignancies. 66 patients had complete response. Non-relapse mortality rates on day 100 were 37.4% for the fludarabine/melphalan combination and 87.5% for the cladribine/melphalan combination. This had caused the latter study arm to close early.

Slavin's group from Jerusalem<sup>16</sup> reported 26 patients with blood disorders who underwent peripheral blood stem cell transplantation up front from HLA- identical sibling using a combination of fludarabine, busulphan and anti-thymocyte globulin as conditioning. The

conditioning regimen is very well tolerated with no severe procedure related toxicity. However severe GVHD (grade 3 and 4) occurred in 6 cases and is the cause of death in 4 patients, occurring after early discontinuation of cyclosporin. Engraftment was documented in all patients, with 9 of 26 patients developing transient mixed chimerism prior to the establishment of full donor chimerism. With an observation period extending over 1 year, 22 of 26 (85%) patients treated by allogeneic non-myeloablative stem cell transplantation are alive and 21 (81%) are disease free. The same group using the same conditioning regimen in 23 patients<sup>17</sup> with lymphoid malignancies obtained a moderate response. Similar encouraging rates of donor engraftment using a preparative regimen combining fludarabine with cyclophosphamide are reported in small series of patients by groups in Bethesda<sup>18</sup> and Genoa, Italy."

Low dose total body irradiation (TBI) based regimen is an alternative approach of delivering non-myeloablative but highly immunosuppressive conditioning. The Seattle group<sup>20</sup> had evaluated non-myeloablative transplantation with low dose TBI (200cGy) without (60 patients) or with (28 patients) fludarabine before HLA-matched sibling peripheral blood stem cell transplant, followed by post-transplantation immunosuppression with cyclosporine and mycophenolate mofetil in a total of 88 patients. There were 10 graft failures when TBI was given alone. Graft failure was prevented with the addition of fludarabine. 37 patients achieved complete response.

The Boston group<sup>21</sup> developed a protocol in which patients were conditioned using cyclophosphamide, thymic irradiation (700cGy) before transplantation and ATG before and after transplantation. They had demonstrated the establishment of durable multilineage mixed chimerism in 4 of 5 patients with refractory NHL undergoing BMT for mismatched family member. 2 patients were in GVHD-free status of complete and partial clinical remission. Subsequently using similar conditioning regimen in 21 patients with hematological malignancies,<sup>22</sup> they reported 8 complete responses and 6 partial responses.

The London group<sup>23</sup> explored a conditioning regimen of fludarabine, melphalan and CAMPATH-1H (anti-CD52) in 44 patients. They had 22 complete responses and 4 partial responses. It is noteworthy that the incidence of chronic GVHD was very low.

The Barcelona group<sup>24</sup> conducted a

**TABLE 1: Results of selected studies on non-myeloablative hemopoietic stem cell transplantation for blood disorders**

Transplant Centre	Conditioning Regimen	Median Age, yr	Median Followup, d	Subjects Studied	Complete Response	Graft Failure	Acute GVHD	Chronic GVHD	Survival and TRM
MD Anderson Cancer Centre, Giral S.[13]	Fludarabine +Daurubicin +Cytosine arabinoside; or 2CDA + Cytosine arabinoside	60	100	15	7 CR	0	3	0	OS=40% DFS=13% TRM=NA
MD Anderson Cancer Centre, Giral S. [15]	Fludarabine (78) + Melphalan; or Cladribine (8) + Melphalan	52	NA	86	66 CR	4	34	21/46	OS=28% DFS=23% TRM=37%
MD Anderson Cancer Centre, Khouri IF. [14]	Fludarabine +Cyclophosphamide +Cisplatinum + Cytosine Arabinoside	61	180	15	8 CR	4	4	2	OS = 47% DFS= NA TRM=NA
Jerusalem, Slavins S [16]	Fludarabine + Busulphan + Anti-thymocyte globulin	34	240	26	21 CR	0	10	9	OS=85% DFS=81% TRM=15%
Jerusalem, Nagler A [17]	Fludarabine + Busulphan + Anti-thymocyte globulin	41	675	23	NA	0	8	4	OS=40% DFS=40% TRM=31%
Seattle, Sandmaier BM [20]	2 Gy TBI + Fludarabine	55	244	88	31 CR	10	41	57	OS=68% DFS=NA TRM=11%
Boston, Spitzer TR [22]	Cyclophosphamide + Anti-thymocyte globulin + Thymic irradiation	44	445	21	8 CR	3	12	NA	OS=52% DFS=33% TRM=10%
London, Kottaridis PD. [23]	Fludarabine + Melphalan + Campath-1H	41	270	44	22 CR	1	2	1	OS=91% DFS=33% TRM=9%
Barcelona, Martino R. [24]	Fludarabine + Melphalan or Busulphan	53	283	76	41 CR	0	21	41/61	OS=60% DFS=55% TRM=20%
Kuala Lumpur, unpublished data	Fludarabine + Cyclophosphamide or Busulphan	33.5	484	12	9 CR	2	5	4	OS=75% DFS=50% TRM=0%

CR = complete response; TBI = total body irradiation; 2CDA= 2-chlorodeoxyadenosine; OS= overall survival; DFS= disease free survival; TRM= treatment-related mortality; Acute GVHD – grade II to IV.

prospective multicentre study on 76 patients using a reduced-intensity conditioning regimen of fludarabine and melphalan or busulphan. The results show low treatment related toxicity, no graft failure and an apparent low risk of acute GVHD. However, a significant proportion of the patients had chronic GVHD.

We have performed allogeneic HSCT on 12 patients with a variety of blood disorders using a conditioning regimen of fludarabine and cyclophosphamide or busulphan. All except two patients showed successful engraftment. There was no treatment-related toxicity at Day 100, and 9 had complete response.

### **ACHIEVEMENTS AND LIMITATIONS OF NON-MYELOABLATIVE CONDITIONING REGIMENS**

In general the non-myeloablative conditioning regimen is well tolerated. The toxicity is much less in comparison with a standard myeloablative regimen. This low toxicity have been confirmed by reports from the non-myeloablative transplant groups from various countries.<sup>13,14,16,17,18,19,20,22,23,24</sup>

It should be viewed in the context of a patient population with a high median age and high risk of transplanted related mortality. Mucositis was mild and many of them maintained oral intake throughout the procedure and did not require parenteral caloric support. Lower incidence of severe veno-occlusive disease of the liver, interstitial pneumonitis and multiorgan failure has been observed in all the studies. No or shorter period of neutropenia has resulted in fewer febrile episodes due to intercurrent infection. Shorter periods of platelet and packed cell dependence have also been demonstrated. These major advantages have made possible the allogeneic non-myeloablative HSCT being performed safely as an out patient basis.<sup>25,26</sup>

Scanty data is available concerning the medium and long term toxicity of these protocols. It has been postulated that these protocols may also help to bypass frequent late complications that result from the combined effects of high dose chemo-radiotherapy in conventional transplant in terms of preservation of fertility and in children, preservation of growth pattern. However it remains unproven. The long term side effects of intensely immunosuppressive nature of some of the conditioning regimens are yet to be seen.

Using a non-myeloablative protocol for induction of a state of mixed chimerism may help reduce the incidence and severity of GVHD.

Slavin *et.al.*<sup>16</sup> has postulated that host hemopoietic cells can veto donor anti-host alloreactivity while donor hemopoietic cells can veto residual alloreactive host cells, hence explaining why mixed chimeras can result in bilateral transplantation tolerance. Most studies have reported a relatively mild acute GVHD after the initial transplant and it was easily controlled with steroid. In fact, the state of stable mixed chimerism has allowed 2 myeloma patients to receive live kidney grafts from their compatible donors without the subsequent life-long immunosuppression therapy.<sup>27</sup>

Chronic GVHD and GVHD post donor lymphocyte infusion were the major problems with this treatment, limiting their application and effectiveness. Approaches to potentially reduce this risk of GVHD without compromising either engraftment or anti-tumor activity are required. The use of **COMPACT-1H**<sup>23</sup> seems very promising in this respect. It is also remains unclear whether donor lymphocyte infusion should be administered on a prophylactic basis or only in the presence of progressive disease. The effective lymphocyte dose in the context of non-myeloablative protocol remains to be determined. In essence, progress in approaches that maximize the effective dose of lymphocytes administered while minimizing the risk of GVHD is therefore central to the success of these programs.

Graft rejection is another issue of concern. The risk of graft rejection is higher because residual recipient immunity is not fully ablated. However, prompt recovery of the autologous hematopoiesis is usually observed in these patients with no major procedure-related toxicity. Relapse after allogeneic non-myeloablative transplantation is potentially reversible by allogeneic cell therapy.<sup>16</sup> However the incidence of graft rejection and relapse rate has to be based on a larger cohort of recipients and longer observation period.

The factors that determine engraftment, chimerism, and response with non-myeloablative allogeneic transplants are currently unknown. It is conceivable that the preparative regimen, the transplants cell dose, tumour burden, immunosuppressive effects of prior therapy and phase of the disease may be the predictors. The optimum low-intensity allogeneic stem cell transplant technique, which maximizes engraftment and graft-versus-malignancy effects while minimizing transplant-related complications, has yet to be defined.

## THE FUTURE

Availability of a relatively safe protocol for adoptive cell therapy using matched allogeneic stem cells and T cells may be beneficial for a larger number of patients in need, with no upper or lower age limit and with concomitant debilitating diseases. There is still debate about the optimum non-myeloablative conditioning regimen for specific patient categories, and new regimens are continually being explored. Finally, it would be necessary to conduct clinical trials comparing a preparative regimen of a non-myeloablative nature with that of a standard one. These trials are being launched in year 2001 and their results would be awaited eagerly.

The graft-versus-malignancy effect inherent in a non-myeloablative HSCT makes this a novel approach in the treatment of metastatic solid tumours. In an ongoing study at NIH<sup>28</sup> on 33 patients with metastatic renal-cell carcinoma, 4 had complete response sustained for at least 19 months, 7 had partial response sustained for at least 8 months. Regression usually coincides with the establishment of 100% donor T-cell chimerism and reduction of immunosuppressive treatment. However, using the same preparative regimen in the treatment of metastatic melanoma, in a series of 17 patients transplanted at the NIH and the Westmead Hospital, Sydney, 15 patients died (2 from treatment-related toxicity and 13 from disease progression). Nevertheless, the promising results in metastatic renal-cell carcinoma have initiated pioneering studies in other metastatic cancers such as breast and prostatic cancers.

Non-myeloablative HSCT has demonstrated high rate of engraftment and reasonable safety even in the risk groups. It is therefore not surprising that it is being extended to non-malignant conditions. Cases of successful transplantation for hemoglobinopathies, severe aplastic anemia, PNH, Diamond-Blackfan syndrome, Fanconi's anemia, chronic granulomatous disease, congenital immunodeficiencies, storage diseases and osteopetrosis have been reported.<sup>16,29,30</sup> It is likely more patients of this nature where low degrees of mixed chimerism may ameliorate the disease would receive this mode of treatment in the future.

Non-myeloablative conditioning regimens have been reported clinically successful in the early report of mismatched related HSCT.<sup>21</sup> It has also been evaluated in mismatch unrelated transplant.<sup>31</sup> Preliminary results have shown a

stable multilineage mixed hemopoietic chimerism with high levels of MHC-incompatible donor cell reconstruction and minimal transplant-related toxicity and mortality in animal study. It may be translated into clinical use in the near future.

Non-myeloablative conditioning regimens may open new avenues in clinical gene therapy. Based on hematopoietic stem cell gene transfer techniques, low level engraftment of genetically modified cells is attainable following conditioning with non-myeloablative low dose irradiation.<sup>32</sup>

Last but not the least, they may serve as a platform for immunotherapy using specific T cell clones against the recipient's disease. Using leukemia-specific T cell clones generated *in vitro* from donor lymphocytes, a patient with relapsed chronic myeloid leukemia and resistant to repeated donor lymphocyte infusions, was successfully induced into remission.<sup>33</sup> In this instance, the leukemia-specific antigen was unknown. However, tumour-specific cytotoxic T cell clones can be generated through some known tumour antigens such as proteinase-3 peptides<sup>34</sup> in chronic myeloid leukemia cells. In this way, a potent anti-tumour effect would be produced without causing GVHD.

## CONCLUSION

Experience to date with these non-myeloablative conditioning protocols are encouraging and they render an allogeneic HSCT effective and safe. Tumour cells or genetically abnormal stem cells may be effectively eliminated by optimal combination of intense immunosuppression with relatively low dose chemotherapy with or without radiotherapy, followed by infusion of donor stem cells enriched with immunocompetent T cells. This procedure induces initial bilateral transplantation tolerance, followed by gradual elimination of all host type cells by donor T cells over time, while controlling for GVHD. The effectiveness and safety of this procedure had been proven in clinical experiments and ready to be exploited in many clinical settings.

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