Non-Myeloablative Stem-Cell Transplantation for Immunotherapy of Cancer and Non-Malignant Diseases with Allogeneic Lymphocytes

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Until recently, autologous as well as allogeneic stem-cell transplantation derived from the marrow compartment, granulocyte colony-stimulating factor-mobilized blood or cord blood sources was based on mandatory myeloablative doses of combinations of chemotherapy or chemoradiotherapy designed to eliminate undesirable cells of host origin (malignant, malfunctioning or genetically abnormal). In preparation for allogeneic bone marrow transplantation, the goal of the conditioning was also to eliminate the immune system cells of the host in order to prevent rejection of the allograft by residual immunocompetent T cells of host origin.

Consequently, BMT was associated with procedure-related toxicity and mortality, which were prohibitive for elderly patients in need of BMT or for patients with poor performance status [1]. Consequently, for many clinicians, the BMT procedure was unacceptable as a modality for treatment of patients at an early stage of their disease. It was used as a last resort for patients exhausting all available alternative options. This meant selecting the worse possible patients with resistant or relapsing disease, frequently heavily beaten by prior cytoreductive chemoradiotherapy and frequently colonized with resistant infectious agents acquired during periods of prior repeated courses of chemotherapy that resulted in neutropenia. Such patients were clearly at high risk to develop complications following exposure to myeloablative chemoradiotherapy, which was further complicated by prolonged pre- and post-transplant immunosuppression due to prophylactic treatment against graft-versus-host disease. GVHD caused further dysregulation of the newly emerging immune system cells derived from uncommitted stem cells or from treatment of established GVHD. Many of the potential problems and existing obstacles can be overcome by using reduced intensity BMT, non-myeloablative stem-cell transplantation or as is frequently called, 'mini-transplant' [2–6]. Consequently, as shown in two reviews in this issue of *IMAJ* [7,8], NST is gradually becoming an acceptable treatment for all patients in need of BMT for both malignant and non-malignant indications [5,6]. For patients not eligible for conventional BMT, such as the elderly or patients with poor performance status [1], NST not only represents the treatment of choice but is most likely the only possible option for cure. In contrast, for patients eligible for conventional BMT, the option of NST exists and seems most attractive, although some still argue that only randomized prospective trials will determine the role of NST as a logical replacement of myeloablative BMT.

NST was conceived in preclinical animal models as early as 1976 [9–14] and pioneered as a clinical modality at the Hadassah University Hospital in Jerusalem in the early 1990s. The rationale for developing this treatment was based on observations dating back to January 1987, starting in a patient who underwent a most aggressive "supra-lethal" conditioning in preparation for BMT from his fully matched sister for resistant acute lymphocytic leukemia [15–17]. Despite the use of maximally tolerated doses of chemoradiotherapy, initial full engraftment with donor cells and initial conversion of host (male) to donor (female) cells, the patient experienced early and aggressive myeloid and extra-myeloid relapse, and normal female cells were replaced with male blasts. At that time we introduced the use of donor lymphocyte infusion in an attempt to induce graft-versus-leukemia effects with alloreactivity against differences in minor histocompatibility antigens, regardless of resistance to chemoradiotherapy, following induction of donor-specific transplantation tolerance. Following DLI, this patient developed mild, self-limited acute GVHD, after which he was again converted to female phenotype [4]. This patient and hundreds of others with a variety of indications for BMT similarly treated in many other transplant centers all over the world, particularly patients with chronic myeloid leukemia [16,18,19], confirmed the efficacy of DLI for the treatment of otherwise incurable blood cancer. Later on, we also documented that DLI could also be applied for displacement of non-malignant hematopoietic cells of host origin in patients with genetic diseases and other non-malignant indications for BMT [20]. Similar to experiments performed in preclinical animal models of human disease, graft-versus-tumor effects, in analogy to GVL effects, were also documented in mice conditioned with total lymphoid irradiation, which is also a non-myeloablative, primarily lymphoablative conditioning [13]. The role of DLI in highly immunosuppressed recipients was then extended and confirmed in patients with lymphoma [21] and metastatic breast cancer [22]. Based on the above, it became obvious that tumor cells fully resistant to myeloablative chemoradiotherapy could be eradicated by DLI, thus suggesting that the hazardous conditioning prior to BMT may be replaced with a much safer lymphoablative procedure, with the goal of inducing host-vs-graft transplantation tolerance by engraftment of donor stem cells. This allows durable engraftment of immunocompetent donor T cells, at the time of grafting or after, for induction of optimal GVL effects at a later stage post-transplantation. We have previously documented that as the interval from BMT to cell therapy increases, the risk of developing GVHD decreases [10]. It appeared that the standard therapeutic strategy in bone marrow transplantation overestimated the anti-cancer potential of high doses of chemotherapy and radiotherapy and underestimated the efficacy of immunotherapy mediated by allogeneic donor lymphocytes, already suggested by Weiden and colleagues in the early 1980s [23,24].

NST was initially pioneered successfully by using cytoxan and anti-T lymphocyte globulin [6] and later on by using various combinations of well-tolerated doses of conventional chemotherapy [2–6], or alternatively, using low dose total body irradiation alone with intensive post-transplant immunosuppression [25,26] or with more intensive pre-transplant lymphoablation with fludarabine [25]. Other investigators chose to perform a tandem transplant, applying autologous bone marrow or blood stem-cell transplantation following high dose chemotherapy for optimal tumor cell debulking, followed by NST for eradication of minimal residual disease [26], especially for multiple myeloma [28]. Thus, NST was based on clinical application of a new therapeutic principle rather than a single well-defined protocol. However, clinical application of NST became most attractive when the use of fludarabine was introduced, because it became apparent that well-tolerated non-myeloablative conditioning could be used for safe engraftment of donor hematopoietic cells, alloreactive lymphocytes included [2–4]. Since then, the use of NST, once acceptable only for patients with definite indication yet not eligible for conventional BMT, is becoming increasingly popular for clinical application for all

BMT = bone marrow transplantation
GVHD = graft-versus-host disease
NST = non-myeloablative stem-cell transplantation
DLI = donor lymphocyte infusion
GVL = graft versus leukemia

indications of BMT [5,6]. However, results must be cautiously interpreted, and prospective randomized clinical trials are certainly indicated to confirm possible advantages of NST over conventional BMT.

With the growing clinical experience using NST for the treatment of acute and chronic leukemia, lymphoma, multiple myeloma and certain genetic diseases, such as enzyme-deficiency disorders, Fanconi's anemia and thalassemia major, it became evident that a similar modality might be applicable for induction of cell-mediated immunotherapy of metastatic solid tumors. Indeed, the first successful application of NST was reported in metastatic renal cell cancer in patients considered incurable, with a remarkable response rate and even durable complete remission [29]. The therapeutic principle used for all indications was the same, including three main phases: pretreatment with immunosuppressive agents to prevent rejection of major histocompatibility class-matched hematopoietic stem cells obtained from a normal sibling or matched unrelated donor; engraftment of donor stem cells preferably obtained by mobilizing blood stem cells, thus containing a large stem-cell inoculum enriched with donor immunocompetent T cells; and allowing donor immunocompetent lymphocytes (present in the graft and/or added later on in the course of DLI) to eradicate all hematopoietic cells of host origin, including tumor cells. The use of DLI may be particularly recommended for patients at risk with no GVHD following discontinuation of post-transplant immunosuppression.

While the results of various NST regimens are most encouraging, one unresolved problem is acute and chronic GVHD, which still remains as a major barrier to more successful outcome. Clearly, defining new approaches for better control of GVHD are urgently needed to improve the already confirmed benefits of NST. Several approaches may enhance the efficacy and reduce the risks of immunotherapy with donor lymphocytes. Recent experiments in our laboratory suggest that anti-cancer effects can be significantly improved while eliminating or reducing the severity of graft-versus-host disease by using immune rather than naïve donor T cells [30–32]. Such immune T cells can be generated *in vitro* by culture of lymphocytes with dendritic cells pulsed with tumor-specific peptides or the patient's tumor cells. Another approach currently under investigation, which could also be applied to patients without a histocompatible sibling, involves the use of T cell-depleted allografts to induce tolerance with no risk of GVHD, followed by administration of graded increments of donor T cells or tumor-specific T cells, with careful titration until elimination of all tumor cells of host origin or until the first evidence of GVHD. Alternatively, donor lymphocytes may be transduced *in vitro* prior to cell therapy by insertion of a suicide gene, such as the herpes simplex virus thymidine kinase gene, for limiting the life span of donor lymphocytes by administration of gancyclovir in cases of uncontrolled GVHD [33].

Although much remains to be done for improving the clinical application of NST, the proof of principle is already confirmed. It seems acceptable that allogeneic alloreactive lymphocytes can eradicate and replace the host with a donor immunohematopoietic system, thus causing curative GVL and occasionally graft-versus-tumor effects, while avoiding the use of myeloablative conditioning, thus avoiding or minimizing procedure-related toxicity and mortality. Future progress in extending the use of NST for a larger number of patients in need, possibly all candidates for BMT, will depend on development of improved strategies for safer and better regulation of cell-mediated immunotherapy independently of anti-host responses.

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