

# Cellular Therapy by Non-Myeloablative Stem Cell Transplantation and Immunotherapy of Minimal Residual Disease

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## Introduction

Allogeneic bone marrow transplantation (BMT) represents the only modality for treatment of hematologic malignancies as well as for replacement of genetically abnormal stem cells with normal donor stem cells, thus presenting an ultimate treatment of choice for patients with many life-threatening malignant and genetic disorders. Treatment-related toxicity and mortality resulting in part from myeloablative chemoradiotherapy still represent a barrier to successful BMT and are prohibitive in considering larger scale clinical application of stem cell transplantation for extending the indications for the treatment of larger number of patients in need, including a variety of genetic diseases that may be correctable by BMT exclusively. We recently introduced a new concept in an attempt to replace myeloablative conditioning with well-tolerated nonmyeloablative conditioning<sup>(1,2)</sup>. Our new protocol in preparation for allogeneic nonmyeloablative stem cell transplantation (NST) is based on the following: (1) We have documented graft-vs-leukemia, graft-vs-lymphoma (GVL) and graft-vs-genetically abnormal stem cell effect in patients with genetic diseases with recurrent disease following BMT<sup>(3-6)</sup>, suggesting that immunocompetent donor lymphocytes may eradicate residual host stem cells, including those resistant to myeloablative doses of chemoradiotherapy<sup>(7,8)</sup>; (2) Complete replacement of malignant as well as normal hematopoietic cells of host origin can be accomplished by nonmyeloablative conditioning, through GVL effects that may be mediated by donor T cells recognizing allogeneic host stem cells and their progeny, resulting in elimination of host immunohematopoietic system, which is being replaced by donor stem cells facilitated by donor T cells. Elimination of all host-type hematopoietic cells by alloreactive donor T cells can be equally or even much more effective than myeloablative conditioning, possibly with minimal procedure-related toxicity and mortality<sup>(1,2)</sup>.

Based on the observations in experimental animals and preliminary clinical studies, we have proposed the use of the same regimen for routine treatment of hematologic malignancies as well as non-malignant diseases, including genetic diseases, using a protocol that focuses on better immunosuppression and less myeloablation, using no radiation at all, aiming for stem cell engraftment for establishing host-vs-graft tolerance to enable durable engraftment of donor alloreactive T cells for elimination of residual hematopoietic stem cells of host origin. Overall, the new trend

is designed towards clinical application of immunotherapy mediated by alloreactive lymphocytes rather than more aggressive chemoradiotherapy.

## Methods

We will present the outcome of more than the first 100 patients who underwent NST for all indications for BMT including acute and chronic leukemia, secondary leukemia, resistant non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD), myelodysplastic syndromes, multiple myeloma, and non-malignant diseases correctable by BMT.

Conditioning consisted of immunosuppression with fludarabine 30mg/m<sup>2</sup>/day x 6 days (Schering AG), rabbit anti-human T lymphocyte globulin 5-10mg/kg (Fresenius AG) and 2 daily oral doses of busulfan 4mg/kg followed by 2 collections of G-CSF (Neupogen, Amgen) mobilized peripheral blood stem cells (5µg twice daily for 5 days). Low-dose cyclosporine A (3 mg/Kg) was used as the only anti-GVHD prophylaxis, starting on day -1, given usually for less than 100 days. The patient with Fanconi's anemia and the patients with severe aplastic anemia were treated with cytoxan instead of busulfan (10mg/g and 60mg/kg on 2 consecutive days, respectively).

## Results and Discussion

The preparatory regimen was well tolerated, with some patients going through the whole transplant procedure with continuous oral caloric intake, with no support of blood products and no septic episodes. All patients featured complete replacement of host to donor hematopoietic cells, occasionally after a transient stage of mixed chimerism, following discontinuation of Cyclosporine A. Day 100 mortality was 4% for standard risk cases (5% malignant diseases and 0% in patients with non-malignant diseases).

Earlier experience in animal models and our cumulative experience in man, suggest that in principle, NST may be an attractive alternative approach for BMT candidates, particularly infants and children with malignant and non-malignant indications for BMT, including genetic diseases with an indication for stem cell transplantation or replacement therapy. Nonmyeloablative conditioning is associated with significantly less immediate and long-term procedure-related toxicity and mortality<sup>(1-7)</sup>. Using NST instead of myeloablative chemoradiotherapy, we anticipate substantially less immediate and long-term side effects as far as growth retardation, multiple endocrine dysfunction and ste-

rility are concerned, especially since normal pregnancy was already observed in one patient with acute leukemia treated similarly<sup>(2)</sup>.

Nonmyeloablative conditioning in preparation for allogeneic stem cell transplantation may thus represent a novel treatment of choice for patients with life-threatening malignant and non-malignant diseases in need of BMT. A larger cohort of patients and longer observation period will be required to assess whether NST will result in better event-free and disease-free survival as compared to conventional myeloablative BMT. Likewise, there is a need to confirm that NST may provide an option for cure for elderly individuals in need of BMT who were not considered eligible until recently due to anticipated unacceptable procedure-related toxicity and mortality.

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