

**BONE MARROW TRANSPLANTATION &  
CANCER IMMUNOTHERAPY  
CELL THERAPY & TRANSPLANTATION  
IMMUNOBIOLOGY RESEARCH CENTER  
ISRAEL'S BONE MARROW  
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## INTRODUCTION

The Department of Bone Marrow Transplantation (BMT) at Hadassah University Hospital serves as Israel's National BMT Center. Presently, approximately 150 BMT procedures are carried out yearly, including autologous and allogeneic bone marrow, blood stem cells and cord blood transplantation procedures for patients with a variety of non-malignant disorders, hematological malignancies and solid tumors. Likewise, the Department is involved in cell therapy for a large variety of marrow deficiency diseases (e.g., aplastic anemia); immune deficiencies (e.g., severe combined immunodeficiency); genetic diseases (e.g., thalassemia major, Gaucher's disease, etc.) and gene therapy (e.g., adhesion deaminase deficiency). The BMT Department operates in conjunction with the Cell Therapy and Cancer Immunobiology Research Centers, and the Danny Cunniff Leukemia Research Laboratory. They are all involved in basic science of cancer, immunotherapy, transplantation immunobiology, gene therapy and stem cell biology, and clinically oriented research.

The Department of Bone Marrow Transplantation has pioneered clinical applications of BMT in Israel. Research activities include adoptive cellular immunotherapy of cancer and tumor cell vaccination, new approaches for autologous and allogeneic BMT, focusing on induction transplantation tolerance to bone marrow and organ allografts, on reduced intensity conditioning and understanding the mechanisms of unresponsiveness, induction of self-tolerance in autoimmune diseases, as well as developing new approaches for enhancing hematopoietic and immunological reconstitution following BMT.

The BMT Department has a positively pressurized GMP-like Cell Therapy Center; a large cryopreservation unit, a leukopheresis and lymphopheresis unit for cellular adoptive immunotherapy, and a cord blood bank. The BMT Department is also in charge of the animal research facility at the Ulman building. Services include outpatient clinics and daycare unit for BMT candidates and recipients, and for cancer immunotherapy, treatment of autoimmune diseases, and all clinical conditions that may be treated with cell or gene therapy.

## RESEARCH AREAS

### Induction of transplantation tolerance to bone marrow and organ allografts

Bone marrow transplantation (BMT) is the treatment of choice for an increasing number of malignant and non-malignant diseases including hematologic malignancies and metastatic solid tumors on the one hand, and a long list of immune deficiencies, genetic diseases, diseases caused by deficiency of stem cell products and a long list of life-threatening autoimmune disorders. In order to enable safer engraftment of bone marrow stem cells for patients with an available matched donor, ongoing research aims to introduce more effective approaches for prevention of rejection, and towards safer clinical applications of BMT for patients with a partial match from a family member (haploidentically mismatched allograft) or matched unrelated donors. Whereas until recently, myeloablative conditioning was considered mandatory, recent data suggest that selective elimination of alloreactive lymphocytes may be a much more effective and safer approach for engraftment of stem cells.

In parallel with using BMT procedure for the treatment of a long list of diseases (as above mentioned) incurable by any other modality, stem cell transplantation allows engraftment of a donor's organs following induction of specific transplantation tolerance, thus avoiding the need for life-long maintenance immunosuppressive treatment. Our goal is to use non-myeloablative stem cell transplantation (NST) across major histocompatibility barriers; this endeavor will facilitate clinical application of stem cell transplantation for induction of transplantation tolerance to organ allografts, and pancreatic stem cells for treatment of Type 1 diabetes. Successful elimination of alloreactive lymphocytes may also allow for the successful engraftment of bone marrow cells and consequently, the use of similar technology for engraftment of cells, tissues and organs from pigs, in a more distant future. We are also developing new approaches for induction of graft-versus-host transplantation tolerance with similar elimination of alloreactive T lymphocytes of donor origin against the host in order to allow for bilateral unresponsiveness of host-versus-graft and graft-versus-host alloreactive lymphocytes.

**Keywords:** Bone marrow transplantation; Stem cell transplantation; Rejection; Graft-versus-host disease; Immunological Unresponsiveness; Transplantation tolerance; Hematologic malignancies; Solid tumors; Cancer; Genetic diseases; Autoimmune diseases; Organ transplantation; Type 1 Diabetes Mellitus.

### Recent Publications

Carella AM., Champlin R., Slavin S., McSweeney P., Strob R. (2000) "Mini-allografts": ongoing trials in humans. *Bone Marrow Transplant.* 25(4):345-50.

Carella AM., Giralt S., Slavin S. (2000) Low intensity regimens with allogeneic hematopoietic stem cell transplantation as treatment of hematologic neoplasia. *Haematologica.* 85(3):304-313.

Nagler A., Slavin S., Varadi G., Naparstek E., Samuel S., Or R. (2000) Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for a malignant lymphoma. *Bone Marrow Transplant.* 25(10):1021-8.

Nagler A., Or R., Naparstek E., Varadi G., Slavin S. (2000) Second allogeneic stem cell transplantation using non-myeloablative conditioning for patients who relapsed or developed secondary malignancies following autologous transplantation. *Experimental Hematol.* 28:1096-1104.

Slavin S., Or R., Aker M., Shapira MY., Panigrahi S., Symeonidis A., Cividalli G., Nagler A. (2001) Nonmyeloablative stem cell transplantation for the treatment of cancer and life-threatening nonmalignant disorders: past accomplishments and future goals. *Cancer Chemotherapy & Pharmacology.* 489(1):S79-S84.

Slavin S., Nagler A. Shapira M., Panigrahi S., Samuel S., Or R. (2001) Non-myeloablative allogeneic stem cell transplantation focusing on immunotherapy of life-threatening malignant and non-malignant diseases. *Critical Reviews in Hematology/Oncology.* 9:25-29.

Nagler A., Aker M., Or R., Naparstek E., Varadi G., Brautbar C., Slavin S. (2001) Low intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. *Experimental Hematol.* 29:362-370.

Slavin S., Nagler A., Aker M., Shapira MY., Cividalli G., Or R. (2001) Non-myeloablative stem cell transplantation and donor lymphocyte infusion for the

treatment of cancer and life-threatening non-malignant disorders. *Reviews in Clinical and Experimental Hematology*. 135-146.

Slavin S. (2002) Non-myeloablative stem cell transplantation for immunotherapy of cancer and non-malignant diseases with allogeneic lymphocytes. *Israel Journal Med Assoc (IMAJ)*. 4(4);284-7.

Slavin S. (2002) Non myeloablative stem cell transplantation. *Biomedical progress*. (In press).

Slavin S., Aker M., Shapira MY., Panigrahi S., Cividalli G., Or R. (2002) Non-myeloablative stem cell transplantation for the treatment of cancer and life-threatening non-malignant disorders: Past accomplishments and future goals. *Transf Apher Sci*. 27(2):159-66.

Or R, Shapira MY, Amar A, Ackerstein A, Samuel S, Aker M, Naparstek E, Nagler A, Slavin S. (2002) Non-myeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. *Blood*. (In press).

Slavin S. (2002) Non-myeloablative stem cell transplantation (NST) for induction of host vs graft tolerance for adoptive immunotherapy of malignant and non malignant diseases and towards transplantation of organ allografts. *Transplantation Proceedings*. (In press).

## Immunotherapy of cancer

A large majority of hematologic malignancies and metastatic solid tumors cannot be eradicated by the available anti-cancer modalities. Some tumor cells are primarily resistant to chemotherapy and radiation, while others develop resistance in the course of cycles of chemotherapy. Eradication of such tumor cells may require an alternative strategy such as immunotherapy. We are currently developing new approaches for cancer immunotherapy based on the use of alloreactive and tumor reactive lymphocytes of donor origin aiming for donor lymphocyte infusion (DLI) after induction of host-versus-graft transplantation tolerance on the one hand, and, on the other, using specifically immune donor lymphocytes generated in vitro or in vivo. In parallel, we are developing new approaches for activation of lymphocytes by cytokines in order to maximize their therapeutic potential. In addition, targeting of anti-cancer effector cells by

monoclonal and tumor-specific antibodies and bispecific antibodies is being investigated. The use of donor lymphocyte infusion for immunotherapy of cancer is applied in conjunction with induction of host-versus-graft transplantation tolerance inducible by non-myeloablative stem cell transplantation (NST).

**Keywords:** Bone marrow transplantation; Non-myeloablative stem cell transplantation (NST); Immunotherapy; Cell therapy; Cytokines; Immune donor lymphocytes; Donor lymphocyte infusion (DLI).

**Recent Publications:**

Slavin S., Or R., Prighozina T., Gurevitch O., Aker M., Panighari S., Shapira M., Nagler A. (2000) Immunotherapy of hematologic malignancies and metastatic solid tumors in experimental animals and man. *Bone Marrow Transplantation*. 25(2):S54-S57.

Morecki S., Yacovlev E., Gelfand Y., Trembovler V., Shoshami E., Slavin S. (2000) Induction of anti-tumor immunity by indomethacin. *Cancer Immunol Immunother*. 48:613-620.

Morecki S., Slavin S. (2000) Toward amplification of a graft vs leukemia (GVL) effect while minimizing graft vs host disease (GVHD). *Journal of Hematotherapy*. 9:355-366.

Nagler A., Ackerstein A., Or R., Naparstek E., Slavin S. (2000) Adoptive immunotherapy with haploidentical allogeneic peripheral blood lymphocytes (PBL) following autologous bone marrow transplantation (ABMT). *Exp Hematol*. 28(11):1225-1231.

Slavin S. (2000) Cancer immunotherapy with alloreactive lymphocytes. *The New England Journal of Medicine*. 343(11):802-803.

Leshem B., Vourka Karussis U., Slavin S. (2000) Correlation between enhancement of graft vs leukemia effects following allogeneic bone marrow transplantation by rIL-2 and increased frequency of cytotoxic t-lymphocyte precursors in murine myeloid leukemia. *Cytokines, Cellular & Molecular therapy*. 6:141-147.

Morecki S., Yacovlev E., Gelfand Y., Uzi I., Slavin S. (2001) Cell therapy with pre-immunized effector cells mismatched for minor histocompatible antigens, in the treatment of a murine mammary carcinoma. *Journal of Immunotherapy*. 24(2):114-121.

Slavin S. (2001) Immunotherapy of cancer with alloreactive lymphocytes. *Lancet Oncology*. 2:491-498.

Slavin S., Ackerstein A., Morecki S., Gelfand Y., Cividalli G. (2001) Immunotherapy of relapsed resistant chronic myelogenous leukemia post allogeneic bone marrow transplantation with alloantigens pulsed donor lymphocytes. *Bone Marrow Transplant*.28:795-798.

Slavin S., Nagler A., Shapira MY., Aker M., Cividalli G., Or R. (2002) Treatment of leukemia by alloreactive lymphocytes and non-myeloablative stem cell transplantation. *Clinical Immunology*. 22(2):64-69.

Slavin S., Morecki S., Weiss L., Or R. (2002) Donor lymphocyte infusion (DLI): the use of alloreactive and tumor reactive lymphocytes for immunotherapy of malignant and non-malignant diseases in conjunction with allogeneic stem cell transplantation. *Journal of Hematotherapy & Stem Cell Research*. 1:265-276.

Prigozhina T., Gurevitch O., Morecki S., Yakovlev E., Elkin G., Slavin S. (2002) Non-myeloablative allogeneic bone marrow transplantation as immunotherapy for hematologic malignancies and metastatic solid tumors in pre-clinical models. *Exp Hematol*.30:89-96.

Slavin S., Morecki S., Weiss L., Shapira MY., Or R. (2002) Immunotherapy of hematologic malignancies and metastatic solid tumors in experimental animals and man. *Critical Reviews in Hematology/Oncology*. (in press).

## Tumor cell vaccines

In order to maximize immunotherapy of cancer, we are currently developing new strategies for tumor cell vaccination, trying to sensitize the patient's as well as donor's own lymphocytes following non-myeloablative conditioning and induction of host-versus-graft transplantation tolerance by Non-myeloablative Stem cell Transplantation (NST). In order to sensitize lymphocytes, we are currently applying in vitro and in vivo approaches, trying to immunize the patient or the donor directly or through manipulations with antigen-presenting cells (dendritic cells) and use them as vaccines using peptides, tumor cell lysates, allogeneic tumor cell lines or donor lymphocytes sensitized against the tumor by mixed lymphocyte tumor cultures.

**Keywords:** Vaccine; Tumor-specific peptides; Tumor cell lysates; Antigen-presenting cells; dendritic cells, Immunization.

## Gene therapy and genetic engineering

We are currently interested in gene therapy of diseases caused by deficiency or abnormality of known genes with vector available. Following the successful cure of a patient with severe combined immune deficiency (bubble baby) with ADA deficiency, we are currently attempting similar trials in other conditions where gene therapy might be beneficial. The availability of the cell therapy technology and the GMP lab facility for cell therapy at our Center allows us to apply gene therapy to a larger number of clinical indications. Current attempts are directed towards insertion of the herpes simplex virus thymidine kinase suicide gene into T lymphocytes in order to eliminate them in case of graft-versus-host disease (GVHD) following cancer immunotherapy with donor lymphocytes. The feasibility to eliminate lymphocytes causing GVHD by using mismatched donor lymphocytes which are much more effective than matched donor lymphocytes for eradication of allogeneic tumor cells, would make it possible to improve our capacity to cure cancer.

**Keywords:** Gene therapy; Suicide gene; ADA gene; Immunodeficiency; Transduction of stem cells.

### Recent Publications:

Hiller C., Wittmann S., Slavin S., Fickenscher H. (2000) Functional long-term thymidine kinase suicide gene expression in T cells using a herpesvirus saimiri vector. *Gene Therapy*.7(8):664-74.

Hiller C., Tamguney G., Stolte N., Matz-Resing K., Lorenzen D., Hor S., Thurau M., Wittmann S., Slavin S., Fickenscher H. (2000) Herpesvirus siamiri pathogenicity enhanced by thymidine kinase of herpes simplex virus. *Virology*.278:445-455.

Aiuti A., Slavin S., Aker M., Ficara F., Deola S., Mortellaro A., Morecki S., Andolfi G, Tabucchi A., Carlucci F., Murinello E., Cattaneo F., Vai S., Servida P., Miniero R., Grazia Roncarolo M., Bordignon C. (2002) Correction of ADA-SCID by stem cell gene therapy combined with non-myeloablative conditioning. *Science* 296;2410-2413.

## Enhancement of the immune system

We are currently searching for new approaches to enhance the function of the immune system following transplantation of stem cells and cancer chemoradiotherapy. In most cases, patients undergoing autologous or allogeneic stem cell transplantation or patients undergoing intensive chemoradiotherapy are immunodeficient for a substantial period of time which may last several years. Facilitation of reconstitution and function of lymphocytes may reduce the risk of overwhelming infections; particular attention is given to IL-7, a crucial cytokine that has already been documented to facilitate the maturation and function of the immune system.

In addition, we study facilitation of function of lymphocytes by combination of IL-7 and IL-2 and other cytokines as well as improving the microenvironment of stem cells as a parameter for improved reconstitution of stem cell proliferation, immune system cells included.

**Keywords:** Immune reconstitution; Immune function; Cytokines; Interleukin 7; Interleukin 2.

### Recent Publications:

Nagler RM., Reznick AZ., Slavin S., Nagler A. (2000) Partial protection of rat parotid glands from irradiation-induced hyposalivation by manganese superoxide dismutase. *Archives of Oral Biology*.45(9).

Ilan Y., Nagler A., Zeira E., Adler R., Slavin S., Shouval D. (2000) Maintenance of immune memory to the hepatitis B envelope protein following adoptive transfer of immunity in bone marrow transplant recipients. *Bone Marrow Transplant*. 26(6):633-8.

Panigrahi S., Nagler A., Or R., Wolf DG., Slavin S., Shapira MY. (2001) Idolent aspergillus arthritis complicating Fludarabine-based non-myeloablative stem cell transplantation. *Bone Marrow Transplantation*. 27(6):659-61.

Weiss L., Barak V., Zeira M., Abdul-Hai A., Raibstein I., Reich S, Hirschfeld E., Gross D., Slavin S. (2002) Cytokine production in Linomide treated NOD mice and the potential role of Th1/Th2 shift on the autoimmune and the anti-inflammatory process. *Cytokine*. (In press).

## Autoimmune diseases

We are currently developing new approaches for the treatment of autoimmune diseases by reinduction of self-tolerance following a two-step procedure:

1. Elimination of host-reactive lymphocytes by chemotherapy and immunotherapy;
2. Reinduction of self-tolerance by infusion of purified autologous or allogeneic stem cells. Pre-clinical animal models of multiple sclerosis (autoimmune encephalitis) and tType 1 diabetes (autoimmune insulinitis in NOD mice) being the primary animal models. In parallel, in collaboration with rheumatologists, we are applying non-myeloablative stem cell transplantation (NST) with autologous or allogeneic stem cell reconstitution in patients with multiple sclerosis and resistant autoimmune diseases.

**Keywords:** Autoimmune disease; Multiple sclerosis; Rheumatoid arthritis; Systemic Lupus Erythematosus; Type 1 diabetes; autologous stem cell transplantation; Allogeneic stem cell transplantation; Non-myeloablative stem cell transplantation.

Stem cell plasticity

### Recent Publications:

Weiss L., Slavin S., Reich S., Cohen P., Shuster S., Stern R., Kaganovsky E., Okon E., Rubinstein AM., Naor D. (2000) Induction of resistance to diabetes in non-obese diabetic mice by targeting CD44 with a specific monoclonal antibody. *Proc. Natl. Acad. Sci. (PNAS)*. (4);97:285-90.

Slavin S., Nagler A., Varadi G., Or R. (2000) Graft vs autoimmune lymphocytes (GVA) following allogeneic bone marrow transplantation in a patient with chronic myelogenous leukemia and severe systemic psoriasis and psoriatic polyarthritis. *Exp Hematol*. 28(7);853-7.

Gross DJ., Weiss L., Reibstein I., Hedlund G., Dalen E., Rapoport M., Slavin S. (2001) The immunomodulator Linomide: Role in treatment and prevention of autoimmune diabetes mellitus. *International Immunopharmacology*. 1131-1139.

Richard K. Burt, Walter Barr, Yu Oyama, Ann Traynor, Shimon Slavin. (2001) Future strategies in hematopoietic stem cell transplantation for rheumatoid arthritis. *J of Rheumatology*.;28(64);42-48.

Burt R.K., Slavin S., Burns W.H., Marmont A.M. (2001) Induction of tolerance in autoimmune diseases by hematopoietic stem cell transplantation: Getting closer to a cure? *Blood*.99(3)768-784.

Weiss L., Abdul-Hai A., Shufaro Y., Reich S., Slavin S. (2002) Linomide administration following bone marrow transplantation in mice. *Cancer Immunol Immunother.* (in press).

## Embryonic Stem Cell

One of the most amazing discoveries of the past few years stems from the documentation of plasticity of stem cells. Embryonic stem cells, naturally, can undergo a physiologic change (differentiation and maturation) to various cells and tissues. Thus, embryonic stem cells may be used as precursor cells for cells and organs once the differentiation and maturation can be controlled in a desirable way. Interestingly, mesangial stem cells can also develop into undifferentiated stem cells and convert, under the proper conditions, into well differentiated cells resembling liver cells, colon cells, epithelial cells, skeletal and myocardial muscles, bone and cartilage. Stem cells may also be derived from cord blood cells. There are many potential indications for stem cell transplantation, and it remains to be seen which of the sources (embryonic stem cells, cord blood stem cells, bone marrow cells or peripheral blood stem cells) may provide the best source for clinical purposes. Obviously, the clinical applications are numerous, and more research is needed to show which functions stem cells can replace. Likewise, transplantation of stem cells from either source may also be beneficial for induction of transplantation tolerance to more mature stem cells from allogeneic and xenogeneic sources.

**Keywords:** Stem cells; Stem cell plasticity; Embryonic stem cells; Mesangial stem cells; Bone Marrow; Cord blood; Blood stem cells.

## Reconstruction of bones and cartilage and creation of microenvironment for hematopoietic stem cells in the bones

We are developing a new approach to facilitate the reconstitution of stem cells by improving the microenvironment of the marrow space, using mixtures of bone marrow stem cells and demineralized bone. Using such a mixture, with the addition of osteoreinductive agents and agents that help create the scaffold for

new bone and joint formation, we can now establish new bones anywhere, of any shape, as well as cartilage and osteo-cartilagenous surfaces in damaged joints. Correction of bone and joints following surgery, degenerative disorders of the joints or the bone or traumatic lesions, will be initiated shortly following approval of our clinical protocol by the Helsinki Committee.

**Keywords:** Bone; Cartilage; Joints; Demineralized bone; Bone microenvironment; Bone marrow stroma.

#### **Recent Publications:**

Levy J., Amar A., Brautbar C., Slavin S., Kapelushnik J. (2000) Transient recovery of endogenous immune function following haploidentical peripheral stem cell transplantation in a patient with severe combined immunodeficiency without evidence of engraftment. *Acta Paediatr.* 89(2):248-50.

Morecki S., Gelfand Y., Nagler A., Or R., Naparstek E., Varadi G., Engelhard D., Ackerstein A., Slavin S. (2001) Immune reconstitution following allogeneic stem cell transplantation in recipients conditioned by low intensity vs myeloablative regimen. *Bone Marrow Transplant.* 28:243-249.

## **Heparanase and potential clinical applications of Heparanase therapy**

We are currently investigating the role of Heparanase in health and disease and the potential clinical applications of Heparanase using animal models of bone marrow transplantation and cancer. We will investigate the role of heparanase on lymphocyte migration in animal models of immunotherapy and autoimmunity as well as in models of transplantation and graft-versus-host disease, focusing on the possible use of heparanase for prevention and/or treatment of pathogenic lesions induced by lymphocytes extravasating from the circulation into the tissues.

**Keywords:** Heparanase; Cancer; Autoimmunity; Cell migration; Cell penetration.

## New drug development program

We are currently developing new drugs for targeted cancer immunotherapy on the one hand, and elimination of reactive lymphocytes on the other. Our aim is to induce selective anti-tumor responses by agents that result in enhanced apoptosis of tumor cells or blocking tumor cells by specific tyrosine kinase inhibitors. In parallel, we are synthesizing and investigating new agents for the elimination of alloreactive lymphocytes. This study is aimed at prevention of rejection, graft-versus-host disease and autoimmune responses by reactive lymphocytes, while leaving naive lymphocytes intact to prevent non-specific immunosuppression. We are mostly focusing on small molecules that may be orally absorbed.

**Keywords:** Cancer selective agents; Immunosuppressive agents; Tyrosine kinase inhibitors; Small molecules.