Chairman:
Ardeshir Ghavamzadeh , MD

Scientific Secretary:
Amir Ali Hamidieh , MD

Secretary Executive:
Marjan Yaghmaie , PhD

Scientific committee member

Ardeshir Ghavamzadeh , MD
Amir Ali Hamidieh , MD
Abbas Hajfathali , MD
Abdolhamid Kajbafzadeh , MD
Abdollah Karimi ,MD
Ahmad Tabatabaii , MD
Ali Ghasemi , MD
Ali Akbar Pourfatholah, PhD
Alireza Shoae-hassani , PhD
Akram Karimi, MD
Amir Abas Hedayati asl , MD
Andrea Bacigalupo , MD
Arash Okazi , MD
Babak Bahar , MD
Bagher Larijani , MD
Christian Chabannon,MD
Gabi Rall
Gholamreza Togeh , MD
Hasan Niknejad ,PhD
Hossein Baharvand ,PhD
Hossein Kamranzadeh , MD
Jafar Ai ,PhD
Jakob R Passweg , MD
Julián Sevilla Navarro ,MD
Kamran Alimoghaddam , MD
Keivan Gohari Moghadam , MD
Mahin Nikogoftar ,PhD
Mahshid Mehdizadeh ,MD
Masoud Arzaghi , MD
Marc Bierings ,MD
Marcos deLima , MD
Marjan Yaghmaie , PhD
Maryam Barkhordar,MD
Maryam Behfar , MD
Mehdi Tabaraie , MD
Mohammad Jahani , MD
Mohammad Vaezi, MD
Mohammadreza Ostadali , MD, PhD
Mohsen Nikbakht ,PhD
Naser Aghdami ,MD, PhD
Nezieh Cereb,MD
Nima rezaee , MD
Reza Malekzadeh , MD
Rupert Handgretinger , MD
Seyed Asadollah Mousavi , MD
Sayed Mahdi Marashi , PhD
Sayed Reza Safaie Nodehy , MD
Shirin Afhami , MD
Shahin Shamsian , MD
Volker Witt , MD
Zahra Ghodsi , MD

The 1st International and 5th National Congress on Stem cells

January 15-17
2015
Tehran
Iran

Contents

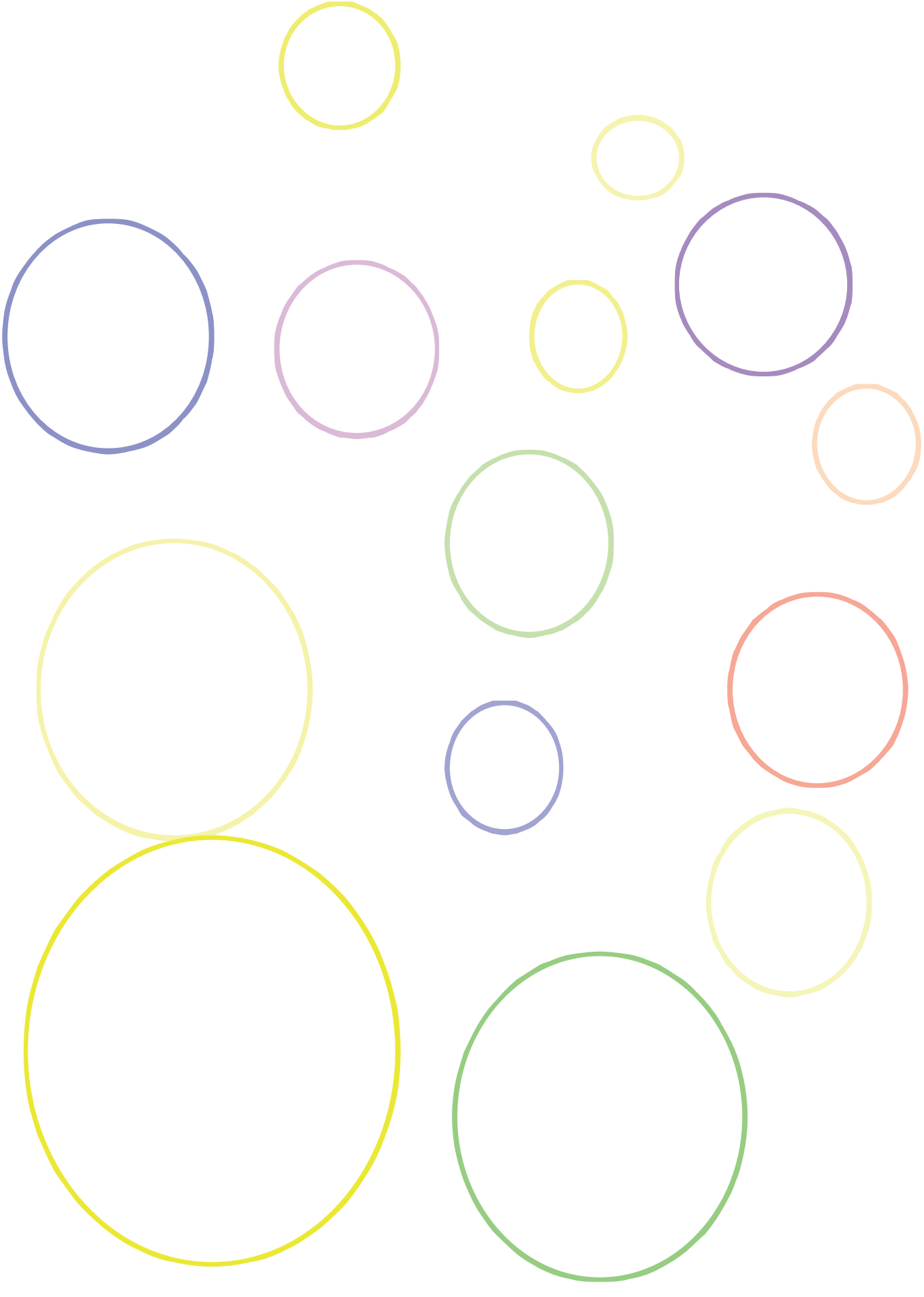
Oral Presentation

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Poster Presentation

The 1st International and 5th National Congress on Stem cells January 15-17, 2015; Tehran , Iran

Oral Peresentation



Donor Factors Affecting Haploidentical Stem Cell Transplantation Outcome

Ostadali Mohammadreza, MD, PhD
Hematology-Oncology and Stem Cell Transplantation Research Center / Tehran University of Medical Sciences,Tehran, Iran.

Several factors affect hematopoietic stem cell transplantation outcome. Some of these are related to the donor including the degree of HLA matching with the patient, donor's age, sex, etc.

In haploidentical transplantation, there are additional factors that presumed to play important roles in the course of treatment.

The phenomenon of fetomaternal microchimerism had been provided a basis for many studies in this field. Based on this fact, many researchers have concluded that haploidentical transplantation between NIMA mismatched siblings (the siblings that have shared paternal haplotype with the patient) are the best haploidentical donors regarding acute and chronic GVHD. The next option is offspring for female patients and the worst ones are paternal donors.

The selection between maternal donors and NIPA mismatched siblings (the siblings with shared maternal haplotype with the patient) is controversial. Van Rood et al. (2002) reported more GVHD and transplant related mortality (TRM) in transplantations from maternal donors but Ichinohe et al. (2004) showed more GVHD in transplantations from NIPA mismatched siblings.

Another active research field in the selection of donors for haploidentical transplantation is NK alloreactivity. It had been shown that NK alloreactivity reduces the occurrence of GVHD, rejection and relapse but the studies in this field have controversial results. It seems that the method of prediction of NK alloreactivity (ligand-ligand model, receptor ligand model, etc) and the transplant protocol (T cell deplete or T cell replete) are the sources of these conflicts.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Stem Cells in Diabetes

Bagher Larijani; MD, F.A.C.E.

Research institute for Endocrine Sciences , Tehran University of Medical Sciences, Tehran. Iran

To date, available treatment options for diabetes which include oral antidiabetic agents and insulin therapy do not provide a cure; they are merely effective in the management of diabetes.However, it is demonstrated that preservation or restoration of pancreatic beta-cell's function can provide a curative treatment for diabetes. Autologous transplantation of adult stem-cells has already been used for treatment of type 1 DM with considerable improvement in daily insulin dose and c-peptide levels. Allogeneic transplantation of adult stem-cells has also been recently used for treatment of diabetes. It is demonstrated that embryonic stem-cells have the highest potential for differentiation into insulin-secreting pancreatic beta-cells and their application does not necessitate immunosuppressive treatment. However, their use is hindered by ethical and legal limitations in many countries. Fetal stem cells which can be obtained from different fetal tissues, including blood, bone marrow, liver and kidney are considered as suitable alternatives to embryonic stem-cells and their use is not as much hindered by limitations imposed on embryonic stem-cells. In this report, I endeavor to provide a brief report of our achievements at the Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences in the field of stem-cell therapy for diabetes. I also deliver a short report of our success in overcoming ethical barriers to stem-cell therapy.

Twenty Four Years of Experience on Stem Cell Transplantation In Iran

Ardeshir Ghavamzadeh, MD

Hematology-Oncology and Stem Cell Transplantation Research Center
Tehran University of Medical Sciences, Tehran, Iran

Aim: From March 1991 through january 2015, a total of 5143 of hematopoietic stem cell transplantations (HSCTs) were performed in the Hematology-Oncology and Stem Cell Transplantation Research Center, affiliated to Tehran University of Medical Sciences. Here we report 24 years of experience in HSCT.
Method: In total, 5143 patients underwent HSCT. Of these transplants, 3279 were allogeneic stem cell transplantation, 1500 autologous stem cell transplantation, and 19 syngeneic stem cell transplantation.The sources of graft were peripheral blood(n=4100), bone marrow(n=516), bone marrow+peripheral blood(n=18) and cord blood(n=66). Ninety-nine patients received mesenchymal cells co-transplantation in addition to HSCT. It is important to point out that the sources of graft in (n=75) allogeneic HSCT were available in our cord blood and stem cell donor program banks.
Result: Stem cell transplantation was performed in patient with leukemias (n=2166), Inherited Abnormality of Red Blood Cell (n=824), Plasma Cell Disorder (n=605), lymphoma (n=629), Severe Aplastic Anemia (n=244), Solid Tumors (n=109), Myelodysplastic(MDS)/ Myeloproliferative (MPS) Diseases (n=87), Disorders of Immune System (n=72), Inherited Disorder of metabolism (n=45), Histiocytic Disorder (n=10) and Auto-Immune Disease (n=5). Morever we had 228 cellular therapies for Postmyocardial Infarction, Multiple Sclerosis, Cirrhosis, Head of Femur Necrosis, Diabetes Mellitus and GvHD treatment. One hundred and thirty-nine autologous transplants have been performed in our institutional outpatient setting. Allogeneic patients were transplanted from HLA- matched identical siblings (n=2886), HLA mismatched [sibling/other relatives] (n=109), syngeneic twins (n=19), HLA matched other relatives (n=178) and unrelated donors (n=107). About 3653 remained alive between 1 to 258 months after HSCT. The main causes of death were relapse, infections, hemorrhagic cystitis and graft versus host disease.
Conclusion: In Iran, HSCT has been successfully adapted in routine clinical care. Recently, new methods such as double cord blood, haploidentical, unrelated and mesenchymal transplantation have been used.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Autologous HSCT for Patients with Autoimmune Disease

Jakob R Passweg, Hematology Division, Basel University Hospital, Switzerland

Within the consortium of the European Group for Blood and Marrow Transplantation approximately 190 transplants are done annually to treat different types of autoimmune disease. Most transplants use autologous peripheral blood stem cells, few are with allogeneic stem cells, mostly patients with hematologic cytopenias e.g. Evans Syndrome. Autologous HSCT is used to treat patients with Multiple Sclerosis, Systemic Sclerosis (SSc), other rheumatoid disease such as systemic lupus erythematosis, juvenile rheumatoid arthritis and inflammatory bowel disease.

Autologous HSCT for severe autoimmune diseases is supposed to eliminate autoreactive T-cells as well as long lived plasma cells and antigen presenting cells and contribute toward tolerance via promotion of regulatory T-cells, restoration of thymic function, reduction of auto-antibodies but also long lasting lymphopenia.

Several observational studies have shown that treatment related mortality was between 1% and 10% according to disease type and that long lasting response was observed in 30%-50% of patients.

Results of 2 randomized studies are available, the ASTIS trial compared autologous HSCT to pulse cyclophosphamide treatment in 156 SSc patients. There was more short term toxicity with HSCT but HSCT conferred a long-term event-free and survival benefit to patients with mainly cutaneous disease. The second is the ASTIC trial in Crohn's disease published as an abstract so far where 45 patients were randomized to receive stem cell mobilization only or stem cell mobilization followed by stem cell transplantation. Significantly more patients in the HSCT group were off immunosuppression and had a Crohn's disease activity index < 150 at follow-up but there was also one death in the HSCT group.

Preventing Acute Leukemia Relapse after Allogeneic Transplants

Marcos Jose Garcia Delima,MD

Section Chief, Stem Cell Transplant Program, Division of Hematology and Oncology, University Hospitals Case Medical Center, Cleveland, OH ,USA

Our field has witnessed a major reduction in transplantation-associated toxicity and mortality. This improvement has paradoxically led to a progressive increase in the overall relapse rates, especially for acute leukemia patients.
In my presentation I will review the issue of leukemia relapse, and discuss approaches to reduce the likelihood of this devastating problem. I will concentrate on the development of low dose azacitidine as a post-transplant maintenance agent, since this will provide us with a frame to discuss difficulties of establishing post-transplant interventions to prevent leukemia recurrence. I plan to also review potential changes in preparative regimen that could lead to less relapses, focusing mostly on acute myelogenous leukemia.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Unmanipulated Haploidentical Transplants Compared with other Alternative Donors and Matched Sibling Grafts

Andrea Bacigalupo, MD

Divisione Ematologia e Trapianto di Midollo, ** Divisione di EmatologiaClinica, IRCCS San Martino, Genova, *Cattedra di Ematologia and ***StatisticaMedica ,Universita' Genova, Italy

We studied 459 consecutive patients with hematologic malignancies, with a median age of 44 years (15-71), grafted from identical siblings (SIB) (n=176), matched unrelated donors (MUD) (n=43), mismatched unrelated donors (mmUD) (n=43), unrelated cord blood (UCB) (n=105) or HLA haploidentical family donors (HAPLO) (n=92). Graft vs host disease (GvHD) prophylaxis consisted of cyclosporine and methotrexate (SIB), with antithymocyte globulin for unrelated donors and UCB, or post-transplant cyclophosphamide, cyclosporine and mycophenolate for HAPLO. Conditioning regimens were mostly myeloablative (69%). Advanced disease phase was more frequent, but not significantly, in HAPLO and mmUD (p=0.08). Acute GvHD grade II-IV was significantly less frequent in HAPLO, UCB and MUD (14-21%) vs SIB (31%) and mmUD (42%) (p<0.001), and there was a trend for less moderate-severe chronic GvHD in HAPLO and UCB (p=0.053). The proportion of patients off cyclocsporine at 1 year, ranged from 55% for SIB to 81% for HAPLO (p<0.001). Transplant related mortality at 2 years, was lower in HAPLO and SIB (18-24%) vs MUD, mmUD and UCB (33-35%) (p=0.1); relapse was comparable in the five groups (p=0.8). The 4 year actuarial survival was 45% (SIB), 43% (MUD), 40% (mmUD), 34% (UCB), 52% (HAPLO) (p=0.1). In multivariate analysis, advanced disease was a negative predictor of survival (HR 2.4, p<0.0001), together with a diagnosis of acute leukemia (HR 1.8, p=0.0001); HAPLO donors were comparable (p=0.8) whereas UCB had inferior survival compared to SIB (p=0.03). In conclusion, unmanipulated haploidentical family donor transplants are an additional option for patients lacking a matched sibling donor. Whether HAPLO donors should be preferred to UCB units, will require confirmatory prospective studies.

The Importance of Hematopoietic Stem Cell Transplantation in Adult Acute Myelogenous Leukemia with Adverse-Risk Karyotype

M.Yaghmaie[1], K.Alimoghaddam[1], M.Jahani[1], SA.Moosavi[1], B.Bahar[1], M.Vaezi[1], H.kamranzadeh[1], A.Ghavamzadeh[1]

[1]Hematology, Oncology and Stem Cell Transplantation Research center, Tehran University of Medical Sciences

Diagnostic karyotype analysis is one of the most classical and powerful independent prognostic indicators in adult acute myeloid leukemia (AML), which serves to identify biologically distinct subsets of disease and has been widely adopted to provide the treatment strategies for each karyotypes. Poor cytogenetic in AML is one of the powerful prognostic factorsat initial diagnosis can be an influential factor for complete remission (CR), relapse, and overall survival (OS), even after HSCT. The coexistence of multiple cytogenetic abnormalitiesdesignated as complex karyotype (CK) has been recognizedas a factor that predicts an extremely unfavorable outcome in AML. The monosomal karyotype (MK), defined as 2 or moredistinct autosomal monosomies or a single autosomal monosomyin the presence of other structural abnormalities, adversely affects the prognosis, and that the overlap of MKwith CK is the main contributor to the unfavorable impact ofCK. Monosomal karyotype remained significantly associated with worse overall survival among patients with unfavorable cytogenetics or complex karyotype, and even inpatients who underwent allogeneic hematopoietic cell transplantationduring first complete remission. These findings confirm that monosomal karyotype has a significantlyadverse effect on post-remission outcome in patients with acute myeloid leukemia treated with and without allogeneichematopoietic cell transplantation in first complete remission. Despite a considerable risk of relapse evenafter transplantation, it is still conceivable that these cytogeneticallyvery unfavorable patients would benefit from allogeneicHCT.Our data confirm that MK exerts a significantlyadverse effect on post-remission outcome in AMLpatients treated with and without allogeneic HCT in CR1.Although our results suggest that allogeneic HCT is alreadyan available treatment of choice, the development of alternativetherapies is warranted for this patient population.The diagnosis of adult AML with adverse-riskkaryotype establishes indications for treatment with intensive chemotherapy followed by allo-HSCT for long-term remission but overall clinical outcomes remain dismalowing to high rates of relapse and therapy-related mortality.Treatment strategies should differ for patients at higher riskfor relapse, but a standard treatment protocol other than allo-HSCT and early reduction of immunosuppressive agentshas yet to be introduced for these patients.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Development of New Malignancies after HSCT: A 23-Year Study

Ardeshir Ghavamzadeh, Kamran Ali Moghaddam, Mohamad Vaezi, Mohamad Jahani, Seied Asadollah Mousavi, Babak Bahar, Hosein Kamranzadeh, Amir Ali Hamidieh, Amir Kasaeian, Akram Karimi.

Hematology Oncology and Stem Cell Transplantation Research Center; Tehran University of Medical Sciences.

Introduction: The patients undergoing HSCT are at a high risk of developing late effects such as new malignancies which strongly affect patients' survival.
Materials and Methods: The study included patients (n=4742) who underwent transplantation between June 22, 1990 and December 10, 2014. During the 23-year follow-up period, 18 patients with malignancies were indentified among HSCT recipients.
Results: Of the 18 patients, 13 were male and 5 were female with a mean age of 40 years (range: 24-75) at the time of transplantation. Mean duration of follow-up post-treatment was 52 months (range: 1-122) for 18 patients. HSCs from peripheral blood are used for all autologous (n=10) and allogeneic (n=8) transplants. HSCTs were used to treat Acute Leukemia (n=7), Multiple Myeloma (n=5), Hodgkin Lymphoma (n=2), Aplastic Anemia, Fanconi Anemia, Chronic Leukemia and DLBCL.
The time interval between diagnosis of new malignancies and HSCT was 44 months. Newly diagnosed malignancies included Sarcoma (n=3), MDS (n=2) and Acute Leukemia (n=2), Gastric cancer, Breast, Spleen, Non-Hodgkin's Lymphoma and Cerebellar Astrocytoma. Eight patients developed relapses and 7 patients died during the study. The causes of death were relapses of disease (n=6) and new malignancy (n=1).
Discussion: The results of the study showed that HSCT recipients are at a high risk of developing new malignancies which affect patients' survival.
Keywords: HSCT, Late Effects, Malignancies

Pediatric Hematopoietic Stem Cell Transplantation provided by Matched Non-sibling Relatives: A new prospect of exploiting extended family search

Amir Ali Hamidieh.MD*,Mohammadreza Ostadali.MD.PhD ,Maryam Behfar.MD, Ashrafsadat Hosseini.BS, Simindokht Basirpanah.BS, Amir kasaeian .PhD, Ardeshir Ghavamzadeh.MD

Hematology, Oncology and Stem Cell Transplantation Research Center, Pediatric Stem Cell Transplant Program; Tehran University of Medical Sciences,Tehran,Iran

Objective: The best donors for hematopoietic stem cell transplantation (HSCT) are full-matched siblings. Inpatientswithout full-matched siblings, HLA registries or cord blood banks are alternative strategies with some restrictions,especially in developing countries.
Methods: Due to high rate of consanguineous marriage in our country, between 2006-2013,extendedfamily search was undertaken in pediatric HSCT candidates with parental consanguinity and no available sibling. Full-matched other- relative donors were found for 93 cases. We retrospectively studiedtheHSCToutcomein these patients. High resolution typing for class I and II alleles was completed for recipient/donor pairs.
Results: The result of search in the extended family was successful in 93 (60male, 33 female) out of 480 of patients (19.3%) who didn't have HLA matched sibling donors. Median age of recipients was 6 years (range: <1-15) at the time of transplantation. The median age of donors was 37 years (range, 9-74). The donors were mother (n = 34), father (n = 23), uncle or aunt (n = 22), grandparents (n = 11) and far related (n = 3). The patients diseases comprised of malignant and non-malignant types. Thalassemia major,Leukemias and Fanconi anemia subgroups had the highest percentage of diseases.The stem cell source was bone marrow in 72 and peripheral blood in 21 patients.
Acute GVHD appeared in 45 patients (48.3%), with 70% of which were of grades I or II. The frequency of aGVHD development in various familial subgroups was not significant. Chronic GVHD developed in 13 patients. The 28-month Overall survival (OS) and Disease-free survival (DFS) were 74% and 69% respectively. No significant difference in OS and DFS was unraveled between various familialrelationships.
Conclusion: Our considerable rate of full matched non-sibling family members and the favorable outcome support the rationale for extended family search in regions where consanguineous marriage is widely practiced. This extensive search should precede seeking cord blood banks and HLA-registries as a time-saving cost-effective alternative strategy.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Pediatric Posttransplant Relapsed/Refractory B-precursor Acute Lymphoblastic Leukemia Shows Durable Remission by Therapy with the T-cell Engaging Bispecific Antibody Blinatumomab

Rupert Handgretinger,

Chair, Children's Hospital, Department of Hematology,Oncology University of Tübingen, Germany

Posttransplant relapsed pediatric patients with B-precursor acute lymphoblastic leukemia (ALL) that were treated with the T-cell engaging CD19/CD3-bispecific single-chain antibody construct blinatumomab on compassionate use. Patients with posttransplant relapsed B-precursor ALL with no further available standard of care therapy were treated with blinatumomab. Blast load was assessed prior to, during and after blinatumomab cycle using flow cytometric based minimal residual disease detection, quantitative polymerase chain reaction for rearrangements of the immunoglobulin or T-cell receptor genes and bcr/abl mutation detection in one patient with Ph^+ ALL. Blinatumomab was administered as a 4-week continuous intravenous infusion at a dosage of 5 or 15 µg/m^2/day. Nine patients received a total of 18 cycles. Four patients achieved complete remission (CR) after the 1st cycle of treatment; two patients showed a CR to the 2nd cycle after previous reduction of blast load by chemotherapy. Three patients did not respond, of whom one patient proceeded to a 2nd cycle without additional chemotherapy and again did not respond. Four patients were successfully retransplanted in molecular remission from haploidentical donors. After a median follow up of 398 days the probability of hematological event-free survival is 30%. Major toxicities were grade 3 seizures in one patient and grade 3 cytokine release syndrome in two patients. In addition to the compassionate use patients, the results of a multicenter phase I/II study will be presenmted and discussed. Blinatumomab can induce molecular remission in pediatric patients with posttransplant relapsed B-precursor ALL and facilitate subsequent allogeneic hematopoietic stem cell transplantation from haploidentical donor with consecutive long-term leukemia free survival.

Extracorporeal Photopheresis: Effective Therapy for Steroid-dependent and Rrefractory Acute Graft versus Host Disease

Volker Witt, MD

St. Anna Children's Hospital,Kinderspitalgasse 6,Vienna ,Austria

Introduction: Extacoproreal photopheresis (ECP) is currently used as second and/or third line therapy for acute and chronic GVHD containing of the collection of MNC/Leukocytes, irradiation with UVA light after adding 8-MOP and then reinfusion to the patient. In our institution we use all three available methods for ECP: 1. Inline method (all 3 steps in one system, UVARXTS™, CELLIX™), offline method (Leukocyte collection by Leukapheresissystem (AMICUS™, OPTIA™,COBEspectra™) and separate irradiation system (Macogenic™)), and MINI method (manual blood drawing, and all steps are manually performed under GMP conditions). Whether there is a difference between the methods no data are available. To elucidate the question, we performed a retrospective analysis of our patients treated for acute GVHD refractory standard immunosuppressive therapy (SIT).
Methods: From 2002 until 2013 23 patients with > grade 2 acute GVHD refractory to SIT were treated with ECP (median age 6.9y (1.5 – 12), median bw 31 kg (7 – 60), 11 f, 12 m). Patients chart were analysed for GVHD, SIT, GVHD treatment, side effects, number of ECP and outcome of acute GVHD. The schedule was performed to our in-house standards. ECP started by a frequency of 2 to 3 reinfusions/week, with individualized tapering due to response of the GVHD and the ability to reduce the immunosuppressive therapy (IT).
Results:In total 338 procedures in 23 patients were enroled (123 inline in 4 patients (bw median 40 kg, median 32ECP(10-49)/patient); 174 offline in 13 patients (bw median 24 kg, median 10 ECP(3-55)/patient); 38 MINI in 6 patients (bw median 10 kg, median 7 ECP(3-9)/patient). No severe side effects were observed in either method. 21/23 patients improved (20 CR, 1 PR (gut), 1 SD (skin+liver), 1 PD (skin, liver, gut)). Log-rank-sum-test showed no statistically significant difference (p = 0.98) between the used methods for outcome and survival. The groups were not comparable to age and bodyweight, therefore a bias has to be claimed. In 22/23 IT could have been reduced, especially corticoid dosage could be tapered.
Conclusion:ECP is in our hands an effective second line therapy in acute GVHD. Neither the method used nor the individualized schedule applied seems to influence the outcome. Due to the small patient number, this report could be only a step forward to prospective randomized trials, bringing hopefully an answer to these questions.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

JACIE Standards for Photopheresis and Apheresis

Julián Sevilla Navarro,MD

Hematología y Hemoterapia Hospital Infantil Universitario Niño Jesús.
Avenida Menéndez Pelayo, 65.28009-Madrid , Spain

The Joint Accreditation Committee-ISCT (Europe) & EBMT (JACIE) was established in 1998 for the purposes of assessment and accreditation in the field of haematopoietic stem cell (HSC) transplantation. The Committee was founded by the European Group for Blood and Marrow Transplantation (EBMT) and the International Society for Cellular Therapy (ISCT), the two leading scientific organisations involved with HSC transplantation in Europe. JACIE in collaboration with the Foundation for the Accreditation of Cellular Therapy (FACT) has established standards for the provision of quality medical and laboratory practice in HSC transplantation.
Last edition of JACIE standards (5th edition, published 2012), for the first time, considers extracorporeal photopheresis (ECP) under the program. Although apheresis for peripheral blood progenitor cell collection had been regulated since the first edition, quality controls regarding ECP were included only in the Clinical Program in that edition. In March next year sixth edition will be delivered, and in this new edition, it will be not only stated under the Clinical Program, but also under the Apheresis Collection Facility Standards.
Through the presentation, the author will discuss different aspects of the standards regarding Apheresis Collection Standards and standard for ECP.

Opportunistic Infections in Hematopoietic Stem Cell Transplantation, A Review Article

Ali Amanati[1*], AbdollahKarimi[1]

[1]Pediatric Infections Research Center, ShahidBeheshti University of Medical Sciences, Tehran, IR Iran
***Corresponding author: Ali Amanati, Pediatric Infections Research Center, Department of Pediatric Infectious Diseases, Mofid Children's Hospital, Dr. Shariati Ave., P.O.BOX: 1546815514, Tehran, IR Iran. Tel/Fax: +98-2122226941, E-mail: ali_amanati_1356@yahoo.com**

Introduction: Advances in supportive care for hematopoietic stem cell transplant (HSCT) recipient results in significant increase in survival and decrease in bone marrow transplant (BMT) related mortality rate over the last decade.[1] Opportunistic infections complicated up to 40 percent of allogeneic HSCTs. [2] Recently, some researcher proposed that certain infections may play a role development of graft-versus-host disease (GVHD). Probiotics may have preventive role and cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) reactivations may accelerated GVHD progression. [3, 4]
Materials (or patients) and methods: In this descriptive study, last published sources of information on infections in HSCT recipient, consisting of books and articles have been reviewed.
Results: Depend on integrity of natural barriers, cell-mediated immunity impairment and phagocytic dysfunction, various range of opportunistic infections may implicated after BMT. Classically, according to risk for development of opportunistic infections, post transplant period divided in three phases.[1, 5] Phase I or Pre-engraftment period which start after transplantation and lasts about 4 to 6 weeks. Neutropenia and barrier breakdown are the major risk factors and gram positive and gram negative bacteria, herpes simplex virus (HSV) as well as Candida species are the most causative agents in this period. Phase II or engraftment period start after phase I and lasts for about 3 months. Impaired cellular and humoral immunity are the major risk factors in this phase. After hematopoietic reconstitution, a severe combined quantitative and functional deficiency in the T and B lymphocyte compartment persists. Immunodeficiency will be prolonged if T-cell depletion has been used or if HLA-incompatibility between recipient and donor exist. Additionally, despite normal white blood cell (WBC) counts, compromised granulocyte functions, primarily impairment of chemotaxis and phagocytosis, may persist. Gram positive bacteria (more commonly in the first half of this phase) and CMV infection are main concern in this period. Epstein-Barr virus (EBV) reactivation is seen frequently in this phase. EBV related post-transplant lymphoproliferative disorder (PTLD) is a complication with high mortality. PTLD more commonly seen in EBV seronegative recipients with EBV seropositive donors and patients having delayed immune reconstitution such as after a T-cell-depleted or HLA-mismatched stem cell transplantation. Candida species, Aspergillus species and Pneumocystis jiroveci

(P. jiroveci) are the most fungal infections in phase II. Phase III or late phase defines as infections occur after third month of BMT. Patients in this phase usually show full hematopoietic and early immune reconstitution. Chronic GVHD and functional asplenia also may play a role. In particular, bacterial infections of the respiratory tract constitute a major cause of death. Life-threatening infections are typically caused by encapsulated bacteria such as Streptococcus pneumoniae or Haemophilus influenza. Varicella zoster infection (VZV) is more commonly seen during the late post-transplant period. Aspergillus species are of the most common fungal infections in phase III. An important pathogen of the late phase after allogeneic stem cell transplantation is P. jiroveci.

Control measures: General recommendation should be considered for all HSCT recipients. These control measures includes: preventing transmission strategy of infections from HCT donors to recipients, complete donor screening (history, physical examination, and review of medical records within 6 months preceding donation), use of potentially safe products, [5] use of HEPA filters, protective isolation hand hygiene and masks by patients, health professionals and visitors. [6] Pre- and post-transplantation vaccination programs should be followed according to Infectious Diseases Society of America (IDSA) [7] and American Academy of Pediatrics (AAP).[8]

Preventing opportunistic infections after HSCT: Preventing Early Disease with antibacterial prophylaxis generally started at the time of stem cell infusion and continued until recovery from neutropenia. Currently, there is no universe consensus regarding antimicrobial prophylactic regimens that can be recommended for children. Other approaches to antibacterial prophylaxis include nonsystemic agents such as Chlorhexidinegluconate (as a skin cleansing product). It has been shown to decrease risk for central line associated infections by at least 50 percent and new acquisition of multidrug-resistant organisms by 30 to 50 percent. [9-12] Growth factors (granulocyte macrophage colony stimulating factor [GM-CSF] and G-CSF) shorten the duration of neutropenia after HCT and may slightly reduce the risk of infection but have not been shown to reduce mortality. [5] Prophylactic administration of fluconazole significantly decreased invasive fungal infections. Fluconazole given until day 75 post-transplant was associated with prolonged protection against invasive candidiasis. [13, 14] Because fluconazole lacks activity against Aspergillus spp. other classes of antifungal agents had been used for prevention of invasive mold infections. Amphotericin B, extended-spectrum triazoles (itraconazole, voriconazole, and posaconazole) and echinocandins had been used with different success rate. P. jiroveci prophylaxis should be initiated at engraftment (or before engraftment, if engraftment is delayed).[8] CMV seropositive HCT recipients and CMV seronegative recipients with a CMV seropositive donor should recieve prevention program from the time of engraftment until at least 100 days after HCT. [5] PTLD high-risk (after T-cell depletion, use of anti T-cell antibodies, umbilical cord transplants, and haplo-identical transplants) patients should be monitor for EBV DNA load for prevention of EBV-related PTLD. Preemptive reduction in immunosuppression and preemptive treatment with rituximab can prevent PTLD.[1, 5, 8]

Discussion: Infection is one of the most important causes of morbidity and mortality after stem cell transplantation. The HSCT recipients are susceptible to various types of bacterial,

viral and fungal infections at different time points after transplantation. Appropriate management of these patients in post-transplant period depends on accurate conception of infections after bone marrow transplantation in each period.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Importance of Adequate Immune Reconstitution after HCT: Tools to Better Predict Immune-Reconstitution

Marc Bierings,MD

Pediatric hematologist, Head, Dept of pediatric hematology and stem cell transplantation, Utrecht University Children's Hospital. Utrecht, The Netherlands.Also on behalf of R Admiraal, C Lindemans and JJ Boelens, Utrecht, The Netherlands.

HCT in children is curative therapy for various malignant as well as inborn diseases of childhood.
Immune recovery after transplant is the result of donor hematopoietic reconstitution. It is of utmost importance to reduce the risk of life-threatening infections as well as the relapse-risk of malignancies. There is however, especially early after transplant, the risk of graft-versus-host disease.
Immune reconstitution is influenced by many factors, eg HLA match, stem cell source and (T) cell dose, serotherapy as well as drugs like ciclosporin,mycophenolatemofetil(MMF) and steroids.
Anti thymocyte globulin (ATG) is widely used as serotherapy. It has well been studied that dose and timing influence engraftment, graft-versus-host risk and immunereconstitution.
This presentation will include data of the influence of timing of serotherapy on outcome in the setting of cord blood transplantation. The impact of dose and timing of ATG on immune reconstitution, viral reactivations and relapse risk will be discussed.
Our centre currently focuses on research with respect to the development of individualized serotherapy to improve immune reconstitution while maintaining acceptable rejection and GvH rates. First results of these modeling studies as well as future prospective study plans will be discussed.
Pharmacological steering of GvHD prophylaxis, using combinations of drugs like tacrolimus, ciclosporin, MMF and steroids can be further refined. To this aim we explore the potential relevance of adapting ciclosporin dose according to area under the curve, as an alternative for trough levels.
Individualised ATG dosing will create a platform to tailor immune-reconstitution, closely monitored in combination with virus reactivation and GvH. Novel ways to treat GvH with limited immune damage, such as mesenchymalstroma cells may further improve rapid immune reconstitution and ultimately survival.

Congenital Neutropenia: Approach from Clinical Phenotypes to Molecular Diagnosis and HSCT Opportunities

Nima Rezaei, MD, PhD

Research Center for Immunodeficiencies, Children's Medical Center; and Molecular Immunology Research Center, Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran

Congenital neutropenia, which is the most common phagocyte defects, could be associated with a number of primary immunodeficiency diseases (PIDs), including oculocutaneous hypopigmentation, pancreatic insufficiency, combined immunodeficiency, metabolic disease, congenital heart disease, and bone marrow aplasia/infiltration. These PIDs consist of several inborn diseases ranging from isolated form of neutropenia such as severe congenital neutropenia (SCN) and cyclic neutropenia to complex inherited disorders associating neutropenia. Although SCN was first described in 1956, mutation in *HAX1* gene, the underlying gene defect for autosomal recessive form of disease, was discovered five decades later. Mutations in some other genes, including *ELANE*, *G6PC3*, *GFI1*,*VPS45*,*JAGN1*, and *WAS* have also been reported in a number of patients with congenital neutropenia. Recombinant G-CSF is the first choice of treatment for neutropenia. G-CSF increases the number of neutrophils and consequently reduces the number of infections and days of hospitalization. However, hematopoietic stem cell transplantation (bone marrow, mobilized peripheral blood stem cells, or cord blood as the source of allogeneic stem cells) is recommended in certain conditions as follow: those who do not respond to G-CSF treatment; those with continuing severe bacterial infections; and those who complicated with development ofmyelodysplasia.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Tuberculosis and HCT

Shirin Afhami

Associate Professor of Infectious Diseases Department Shariati Hospital
Tehran University of Medical Sciences (TUMS)Tehran-Iran

Tuberculosis (TB) is uncommon among HCT recipients. It is 10–40 times more common in recipients of stem cell transplantation than in the general population and varies considerably according to the type of transplant and the geographical location. The incidence of *M. tuberculosis* infections in recipients of allogeneic stem cell trans-plantation ranges between <1 (in the USA) and as high as 16% in the Asia (Pakistan). Patients with prolonged immunosuppression, such as those with chronic graft-versus-host disease (cGVHD), remain at risk for TB, and most cases of TB have occurred in allogeneic HCT recipients (80%), although 20% have occurred in autologous recipients.

In recipients of HCT, the lung is the most commonly involved organ and lung involvement has been reported in 50–100% of allografts. The interval between transplantation and the diagnosis of TB infection varies considerably from as early as 21 days to as late as 3 years post-transplant

Evaluation for latent or active TB in patients who are candidates for HCT should include a history of prior active TB, prior exposure—evaluate as high-priority contacts, results of previous tuberculin skin tests (TSTs) or interferon-gamma release assays (IGRA). Interpretation of the TST may be complicated by a history of prior Bacillus Calmette-Guerin (BCG) vaccination, although tuberculin reactivity following BCG tends to wane over time. Because of prior chemotherapy-induced immunosuppression, the TST is not as sensitive in HCT candidates. IGRAs are specific for TB, but a negative test does not exclude latent TB infection, particularly in the immunocompromised patient. IGRA has been found to be more sensitive than TST in immunocompromised patients suspected of having TB.

Any patient with a recently positive TST or IGRA or a history of a positive test and no prior preventive therapy, should be evaluated for active TB. At a minimum, the patient should be asked about symptoms of systemic disease and respiratory symptoms such as cough and shortness of breath, and a chest radiograph should be assessed. If active TB is detected, therapy and appropriate isolation should be initiated. HCT should be delayed until the active infection is deemed controlled. If the TST or IGRA is positive, but no active TB identified, treatment for latent TB infection should be initiated but the HCT need not be delayed.

INH is well tolerated post-HCT even with concurrent fluconazole use, but concurrent use with itraconazole is not recommended. INH with pyridoxine should be continued for at least 9 months. INH can be started at the completion of conditioning therapy, or prior to conditioning if feasible or if the clinical situation puts the patient at higher risk of infection. The potential for substantial drug-drug interactions between rifampin and immunosuppressive and other agents generally makes this option impractical.

The combination of pyrazinamide and rifampin (PZA/RIF) has known significant liver toxicity and its use post-HCT is not recommended.
Since there is no definitive test to exclude TB, a high index of suspicion should be maintained in recipients of stem cell transplantation living in endemic areas and presenting with compatible clinical and radiological manifestations.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Pulmonary Complications Following Bone Marrow Transplantation

K.GohariMoghadam MD

Associate professor of Tehran University of Medical sciences. Shariati Hospital Pulmonary ward.

Pulmonary complications following Bone Marrow Transplantation (BMT) are important cause of morbidity and mortality of these patients in hospital and after discharge .They need immediate work up and diagnosis and if there is a specific treatment. As a pulmonologist usually we involve in consultation, which requests a specific diagnosis and treatment. Sometimes the condition is so bad that we could not proceed to invasive diagnostic and specific treatments, so we only ordered conservative management, which is common among the many cases of respiratory failure, such as fluid restriction, mechanical ventilation with low tidal volumes and diuretics.

These complications can be categorized to allogeneic vs.autollogous, early vs. late, infectious vs. noninfectious, Pre engraftment, engraftment and post engraftment.

Some complications such as CGVHD and Bronchiolitis Obliterans are much more common than autollogous and the reverse is true about Diffuse Alveolar Hemorrhage.

In the pre en graftment period the major complications are drug toxicities from chemotherapeutic and radiation agents, pulmonary edema either cardiogenic or non-cardiogenic, aspiration, hospital acquired pneumonia.

In engraftment period, engraftment syndrome due to capillary leak is important.

In post engraftment syndrome opportunistic infections, idiopathic pneumonia, Diffuse Alveolar Hemorrhage, Acute GVHD, and after 100 days Bronchiolitis Obliterans are important.

Mainly for diagnosing these complications we need sophisticated and sometimes invasive work up. Some limitations such as very poor condition of the patients, severe thrombocytopenia and ventilator dependency precludes invasive diagnostic methods for abtaining tissue samples.

Approach to Patients with Pulmonary Complications after HSCT

S. Ahmad Tabatabaii;[1]GH Khanbabaii;[1]S.Sadr[1]

[1]Pediatric Pulmonologist; ShahidBeheshti Medical University, Mofid's Children Hospital, pediatric pulmonary ward

Significant morbidity and mortality may occur with Pulmonary complications after HSCT. The best diagnostic approaches depend on the history and physical examination, radiological studies, sputum examination, and serology. Sometime invasive techniques, such as bronchoscopy and surgical lung biopsy, may be necessary.

If the patient is symptomatic (cough, dyspnea, +/- fever) a careful histories and physical examinations are very important in the evaluation of HSCT recipients. The high risk of developing infectious complications from common organisms and opportunistic pathogens and prophylactic antibiotics should be considered. Oral mucositis, narcotics usage and the risk of aspiration pneumonitis are very important. Attention to pleural effusions and/or right upper quadrant tenderness with ascites, sinusitis or pleuritic chest pains with or without hemoptysis ,wheezing before and after HSCT, corticosteroids usage, donor type (unrelated vs sibling), HLA match/mismatch, use of TBI or BU, history of donor lymphocyte infusion, donor and patient gender (female donor—male recipient), patient age, CMV seropositivity of donor and recipient, history of smoking, allergies, chronic infectious complications, lung disease or thoracic irradiation pre-HSCT, history of acute lung injury following allo-HSCT, history of cGVHD at any other site, complete WBC count including differential for eosinophilia and serum Ig levels (IgG and IgA).The time of HSCT [neutropenic phase(30 days), early(1-3 months) and late post engraftment phase] is important in considering lung involvement.

The next step is Chest XR /thorax CT. If pneumonic infiltrates is present (fungal, bacterial, viral) infection is the best diagnosis and BAL is helpful, then the most appropriate treatment should be in the plan. When CXR/CT shows non-specific infiltrates or is Normal, then PFT is necessary. Extrapulmonary cause should be in mind when PFTs are normal too. HRCT and BAL can help If PFTs are impaired. Patchy consolidation and or ground glass attenuation in HRCT with a restrictive PFT may suggest the need for transbronchial biopsy and if nondiagnostic, transthoracic/open lung biopsy. The histologic findings could reveal infection or BO/BOOP/ Late IPS/BOS/COP. If HRCT show air-trapping, small airway thickening or bronchiectasis, and an obstructive PFT is present then histologic test is an obligate, ***but the decision, whether a transbronchial or an open lung biopsy may be performed, has to be made carefully on a case by case basis in the context of radiographical findings, the risk of potential complications and the expected clinical consequences to be made depending upon biopsy results***.

Therapeutic approach may be as follow:

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

If: Airflow obstruction andNo or mild clinical symptoms plus FEV1 drop >5% plus FEV1/FVC < 0.8 or FEV1 <80% with FEV1/FVC > 0.7and no air trappingin HRCTthe best treatments are *Topical Long-acting bronchodilator+ Inhalative corticosteroid* or *Inhalative corticosteroid + montelukast.*

After 2–4 weeks if PFT reassessment improved, then continue topical treatment until PFT become normal or stable for at least 3 months, then slow tapering the medication and PFT monitoring is necessary for every 4 weeks. When there is no change or decline in PFT, then systemic immunosuppression prednisolone 1mg/kg body weight plus continuation of other immunosuppressive therapy may be considered.

If : BO/BOS or COP/BOOP is the best diagnosis then systemic immunosuppression such as prednisolone plus continuation of other immunosuppressive therapy is the choice. Reassessment for COP/BOOP are PFT and HRCT every 2 weeks and for BO/BOS are PFT and HRCT after 4-8 weeks. If PFT stable or improved then taper prednisolone over 3–6 months.And when PFT decline, 2nd and 3rd line immunosuppression plus routinely azithromycin or extracorporeal photopheresis (mTOR inhibitors, CNI, MMF, tyrosine kinase inhibitor, etanercept).

Hemorrhagic Cystitis Complicating Bone Marrow Transplantation in Children: New Concepts and Novel Urological Management

Abdolhamid Kajbafzadeh.MD1*, Amir Ali Hamidieh.MD2,N. Mahmoudi1

[1]Pediatric Urology Research Center, Section of Tissue Engineering and Stem Cell Therapy, Children's Hospital Medical Center, Pediatric Center of Excellence, Tehran University of Medical Sciences, [2]Hematology-Oncology and SCT Research Center Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran (IRI)

Hemorrhagic cystitis (HC) is a major cause of morbidity following bone marrow transplantation (BMT) mostly associated with BK virus infection. The purpose of this presentation is to assess the role of uroflowmetry, conceivable secondary bladder neck dysfunction and to present a novel medical management for this complication in pediatrics age group.

Hemorrhagic cystitis (HC) has been considered as a well-documented complication of pediatric (BMT). Sudden commencement of gross hematuria, frequency, and dysuria in children are the most common symptoms of acute HC. This complication can cause prolonged hospitalization and life threatening morbidity. Early diagnosis and prompted management as well as urinary virus monitoring should be implemented in patients following BMT. Painless microscopic/gross hematuria is the initial presentation, diffuse bladder hemorrhage may lead to clot formation or obstructive uropathy or renal failure. Ten to 68% of BMT cases may present with HC after BMT. Bone marrow transplantation at aging, viral infections, allogeneic transplantation, graft-versus-host disease (GVHD) are the reported predisposing factors. Acrolein as the urinary metabolite of cyclophosphamide and ifosfamide has been considered to be primarily responsible for the occurrence of early HC which occur within 72 h of preparative regimens. The routine application of Mesna (2-mercaptoethanesulfonic acid), as the classical acrolein chemical inhibitor, and hyper hydration can reduce the incidence of early HC by forcing diuresis with or without bladder irrigation and neutralizing the toxicity of the cyclophosphamide-metabolite acrolein. Several studies have developed animal models and future perspectives have discussed the management of HC. Permanent bladder irrigation by evacuating the clots, intravesical bladder instillation of aluminum or formalin, fulguration of bleeding sites and biopsies of suspicious areas, and hyperbaric oxygen therapy are among the alternative treatment modalities of hemorrhagic cystitis. Due to the fact that the accurate mechanism of the HC development is still unclear and challenging, pediatric urologist should take into consideration and alert HC as the significant complication after BMT. Any bladder and urethral catheterization / instrumentation, performing observational cystoscopy, forceful

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

irrigation could increase the high burden of BK viremia (>10^9 copies/mL). However, future insights in the occurrence of HC are expected to shed light which hopefully allow urologists to minimize the consequences of this complication after BMT. The morbidity, logistical, and financial implications of this complication should not be undervalued considering the fact that it may need several urological noninvasive interventions, screening protocols, and new uro-protective drugs.

The 1st International and 5th National Congress on Stem cells
January 15-17, 2015; Tehran , Iran

Cytomegalovirus and Hematopoietic Stem Cell Transplant Patients

Sayed Mahdi Marashi, PhD

Assistant Professor, Virology Department, School of Public Health; Tehran University of Medical Sciences; Tehran, Iran

Background:Human cytomegalovirus like other members of herpes family establishes lifelong latency within the host and as a result, the ability to reactivate from latency is a common feature of this virus. HCMV disease can follow primary infection or reactivation. In immunocompetent individuals, primary (and recurrent) infection with HCMV is usually asymptomatic, indicating a healthy immune system has the ability to control virus. However, when the host immune system is unable to function properly due to either infection such or through iatrogenic intervention following organ transplantation such as hematopoietic stem cell transplant (HSCT) patients, HCMV becomes potentially pathogenic. It has also been demonstrated that there is a direct correlation between peak viral loads and HCMV disease. Accordingly, high levels of virus replication in the T cell immunocompromised patients such as organ transplant patients has been observed to be related with a variety of end-organ diseases caused by HCMV.

The impact of HCMV on the outcome of organ transplant patients is enormous as the virus not only causes high morbidity and mortality but also indirectly influences other relevant outcomes, such as risk of other opportunistic infections. Because of the magnitude of its direct and indirect impacts, there have been extraordinary efforts aimed at defining strategies for its prevention and treatment.In HSCT patients, HCMV has been associated with broad range of clinical complications including pneumonia, gastroenteritis, retinitis, hepatitis, nephritis, and encephalitis.The doubling time of HCMV in healthy subjects is roughly 1-2 days. However, immunosuppressed patients have an even shorter doubling time, so early detection is vital in order to initiate treatment prior to the development of significant disease. Nucleic acid amplification tests are helpful clinically in assessing the risk of disease, ensuring sensitive and rapid diagnosis, and monitoring therapeutic responses in the organ transplant patients. Although viral load testing has largely considered for the diagnosis, prevention, and treatment of HCMV disease, the interpretation of viral load results remains highly complex. This is due to the heterogeneity of patients, variability of the risks within each organ transplant group, differing patient immune profiles and immunosuppressive regimens, and lack of standardization among the many molecular assays used for DNA quantification. Without standardized tests available across different laboratories, clinicians must establish institution-specific viral load thresholds that correlate to the test, patient groups, and disease characteristics.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

HSCT in multiple myeloma patients

Hossein Kamranzadeh.MD[1]

[1]**Tehran University of Medical Sciences, Hematology- oncology and SCT research center , Tehran, Iran, Islamic Republic Of**

Aim: Although autologos HSCT is standard of care for myeloma patients but allogenic HSCT may be the only curable treatment.
Methods: There is 557 patients who underwent HSCT in our center. The conditioning regimen was melphalan for autologos and melphalan+fludarabine for allogenic HSCT. MTX+Cyclosporine A were used as GVHD prophylaxis.
Results: 348 male,209 female resived HSCT. Mean age at transplant is 51 years (range 24-72). 489 autologos and 68 allogenic HSCT performed. Median follow up time is 27.7 months in autologos and 27.2 months in allogenic group. 17.4% died (1.9%in allogenic, 15.5% in autologos). The major cause of death was relapse. 31.3% of autologos and 10.2% of allogenic transplanted patients relapsed. Mean time to relapse is 24.7 months. In allogenic group 36% had acute and 31.34% had chronic GVHD.
Conclusion: Relapse rate is lower in allogenic HSCT (P=0.01), but there is no difference in overall mortality rate. Acute or chronic GVHD had no relationship with relapse rate and it may be due to short time of follow up and few patients number in allogenic group.

Does Additional Chemotherapy Before Allogen HSCT Improve Outcome?

Mohammad Vaezi[1]

[1]Tehran University of Medical Sciences, Hematology- oncology and SCT research center, Tehran, Iran

Acute myelogenous leukemia(AML) is a malignant disease of bone marrow elements. Standard treatment is induction therapy with Cytosine arabinoside and anthracycline based chemotherapy. According to risk group high dose chemotherapy and allogenic hematopoietic stem cell transplantation (HSCT) are used as consolidation in favorable and non-favorable patients respectively. whether consolidation chemotherapy before allogenic stem cell transplantation improves outcome or not is a dilemma.so we allocated patients with complete remission after induction chemotherapy with 7+3 (cytosine arabinoside for 7 days+ daunorubicin for 3 days) standard regimen to two groups. The first group received one course consolidation “5+2” (cytosinearabinoside for 5 days+ daunorubicin for 2 days) regimen then underwent allogenic HSCT, while second group received allogenic HSCT without any additional chemotherapy.
Considering disease free survival (DFS), overall survival (OS) there were not anystatistically significant difference. Additional chemotherapy,probable toxicity or complication and cost favor HSCT without any consolidation. Albeit should be remembered that in this setting patients were sent for transplantation immediately.
We recommend that if AmLpatients are candidate for HSCT, it should be done as soon as possible without any additional chemotherapy after complete remission.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Post-Transplantation Cyclophosphamide as Single-Agent Graft-versus-Host Disease Prophylaxis after Allogeneic Bone Marrow Transplantation Using Myeloablative Busulfan and Fludarabine Conditioning

Marcos Jose Garcia Delima,MD

Section Chief, Stem Cell Transplant Program, Division of Hematology and Oncology, University Hospitals Case Medical Center, Cleveland, OH ,USA

Graft-versus-host disease and preparative regimen toxicity are major causes of treatment failure after allogeneic hematopoietic stem cell transplantation.
The reduced-toxicity myeloablative conditioning regimen of Busulfan (Bu)/fludarabine (Flu) is associated with low rates of non-relapse mortality, while results of post-transplant Cy are remarkable in that it prevents both acute and chronic GVHD. We then conducted a three-institution study (conducted by the groups at MD Anderson Cancer Center, Seattle, and Johns Hopkins) combining the BuFlu regimen with high-dose, post-transplantation cyclophosphamide (Cy). Eligible were patients with human leukocyte antigen (HLA) - matched related and unrelated donors with high-risk hematologic malignancies. Ninety-two adult patients (median age, 49 years; range, 21 to 65 years) were enrolled. GVHD prophylaxis was solely with Cy at 50 mg/kg/day on transplantation days +3 and +4. Cumulative incidences of grades II-IV acute, grades III-IV acute, and chronic GVHD were 51%, 15%, and 14%, respectively. One hundred day and 1 year non-relapse mortality were 9% and 16%, respectively. Several patients received no calcineurin inhibitors during the transplant course.
In my presentation I will discuss these results, and also review the use of post-transplant Cy for GVHD prevention as proposed by the group at Johns Hopkins.

HaploidenticalTransplantation: The Search for the Best Donor

Rupert Handgretinger ,MD

University of Tuebingen, Department of General Paediatrics, Oncology&Haematology, Tuebingen, .Germany

Donor selection in HLA-haplotype mismatched stem cells transplantation might have a significant impact on the development of acute and chronic Graft-versus-Host Disease (GvHD), Transplant-related mortality (TRM), incidence of relapse and overall survival. In contrast to transplantation from allogeneic unrelated donors, where the major focus is on identifying the best HLA-matched donor in order to achieve optimal outcomes, other factors might be more importance in HLA-mismatched transplants from haploidentical donors. These factors might depend on the way haploidentical transplantation are performed, i.e with or without in vitro T-cell depletion. In in vitro T-cell depleted transplantatin using mobilised peripheral stem cells (PBSC) grafts, especially the Killer immunoglobuline-like receptor (KIR) system seems to play an important role in defining alloreactivity of donor-derived Natural Killer (NK) cells against recipients' leukemic blasts. By employing different models to determine NK alloreactivity, a significant impact especially on the incidence of relapse in patients with leukemia has been observed. In T-replete haploidentical transplantation, the effect of alloreactive NK cells might be overridden by alloreactive T-cells requiring intensive pharmacologic GvHD prophylaxis, although an important role of the donor KIR repertoire was reported in patients after T-replete haploidentical transplantation using post-transplant cyclophosphamid. More recent data in T-replete haploidenticaltransplantion using the Bejing approach suggest a role of donor age and younger donors (< 30 years) were associated with a lower incidence of acute GvHD than older donors (> 30 years) and younger male donors were associated with less TRM and better survival than older or female donors. Current and future strategies for the best haploidentical donor identification will depend on the transplant approach employed by the transplant centers and will be discussed.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Extracorporeal Photopheresis for Chronic Graft-versus-Host Disease

Volker Witt, MD

St. Anna Children's Hospital,Kinderspitalgasse 6,Vienna.Austria

Chronic graft-versus-host disease (cGVHD) is serious complication of allogeneic hematopoietic stem cell transplantation (HSCT), and the main course of late non-relapse mortality and morbidity in all age groups, and deleteriously affects the quality of life in surviving patients who otherwise have been cured of their underlying disease. Therapeutic interventions are consisting of immunosuppressive therapies of which corticoids are still the first line treatment option. Due to the fact, that mainly infectious complications are leading to morbidity and mortality, treatment options with no or less immunosuppressive side effects and corticoid sparing effects are urgently needed, in order to ameliorate the outcome of patients with chronic GVHD. Extracorporeal Photochemotherapy (ECP) was introduced in the nineties of the last century to the repertoire of second line treatments for cGVHD (Owsianowski, 1994). Most of the studies for ECP in the treatment of are performed for corticoid refractory cases. Only a few of these were prospectively designed. The major problem is still the definition of what is “steroid refractory”. One possible definition could be: “Salvage treatment should be initiates within 1 month whenever clinical manifestations of cGVHD show evidence of progression in a previously involved organ or whenever clinical manifestations appaear in an organ that was not previously involved.” or “within 3 months, if cGVHD shows no improvement during treatment”. ECP has been shown to be one of few second line treatments, which is able to offer a corticoid sparing effect for treated patients, having at the same time nearly no relevant side effects, with the exception that a venous access is needed and in rare case a central venous access is required. A consensus paper from the EBMT working group defined a recommendation grade of B II for ECP in chronic GVHD, therefore ECP should be offered to steroid refractory cases and “well designed studies are supporting the efficacy of ECP in the second line treatment of cGVHD.In our own institution we could show that patients with corticoid refractory cGVHD, treated with ECP show mainly a corticoid sparing effect, and patients who respond to ECP treatment had a significant better overall survival rate.

Optimizationof Stem Cell Mobilization

Christian Chabannon,MD

Cell Therapy Facility. Institut Paoli-Calmettes. Comprehensive Cancer Centre & Aix-Marseille Université&Inserm CBT-1409. Centre de Recherches en Cancérologie de Marseille. France.

Hematopoietic stem and progenitor cells naturally traffic in and out of the bone marrow. Various types of marrow niches “shelter” hematopoietic stem cells and contribute to the maintenance of stemness through complex cellular and molecular interactions. Some of these interactions are targetable, producing enhanced mobilization to therapeutically useful levels. High numbers of circulating CD34+ cells are observed during the narrow time-window when neutrophils recover after administration of acutely myelo-suppressive agents. rhG-CSF administration also produces an increase in peripheral blood CD34+ cells. These two situations are nowadays routinely exploited in the context of hematopoietic stem cell transplantation, which lead to a major technological switch in medical practices over the last two decades, with the exclusive or predominant use of blood grafts in the autologous and allogeneic settings respectively. Nevertheless, patients and donors who receive mobilization treatments display inter-individual variability, and some of them behave as poor-mobilizers. Plerixafor, the first of its kind agent to target an essential mechanism in stem cell – stromal cell interactions i.e. interaction of the chemokine CXCL12 with its main ligand CXCR4, was recently introduced in the pharmacopeia. Plerixafor can be used in patients affected with lymphoid malignancies who are candidates for high-dose chemotherapy supported with autologous transplantation, when these patients mobilize poorly. Growing experience with this newly available agent confirms its efficacy, even in patients with very low circulating CD34+ cells following administration of rhG-CSF- or chemotherapy-based mobilization regimens. On-demand introduction of plerixafor is increasinglyseen as an alternative to remobilization, either based on the administration of unchanged and repeated mobilization regimen or on the use of specific cyclophosphamide containing mobilization regimen. Beyond medical outcome, the evaluation of the respective merits of plerixafor and other available measures offer an opportunity to streamline a complex medical procedure, optimize the use of hospital resources and eventually contain costs.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Hematopoietic Stem Cell Transplantation for Relapsed or Refractory Hodgkin Lymphoma. A Single Center Experience

Mahshid Mehdizadeh,MD*. Abbas Hajifathali.MD

Taleghani BMT center ,Shahid Beheshti university of medical sciences, Tehran.Iran

Autologous stem cell transplantation (ASCT) is the optimal treatment strategy for Hodgkin lymphoma (HL) patients unresponsive to first course of therapy or relapsing after primary treatment. We analyzed our registry data for patients with Hodgkine lymphoma in Taleghani bone marrow center affiliated to Shahid Beheshti university of medical sciences All the patients with HL who underwent a first autologous or a first or second allogeneic HSCT between 1386/6(2007) and 1392/4(2013) were included. Autologous and allogeneic HSCT were performed in 96and 6 patients, respectively. Three years Overall survival (3yOS) and event free survival was 83% and 67% respectively. We performed allogenic reduced intensity transplantation in 6 refractory or post autologous HSCT relapse. Out of them 3 is in complete remission or stable disease. Grade 2 acute GVHD occurred in one patient who relapsed later and chronic GVHD was also occurred in one patient who is in CR.16 patient passed away, 11 out of them were refractory to autologous HSCT. Disease status at transplantation, disease stage at last relapse, the number of previous chemotherapy courses and age were prognostic factors. OS was favorable even in patients who underwent autologous HSCT in disease status other than complete remission.A first allogeneic HSCT without a previous autologous HSCT was performed only in one patient who relapsed early even after transplantation. In conclusion, autologous HSCT is effective and even curative in patients with relapsed and refractory HL It is preferred to performedas soon as possible in patients who responded to salvage chemotherapy. Allogeneic HSCT is feasible with little complications and might be beneficial in patients who relapsed after autologous HSCT.

CML Transplantation: Iran Experience

Babak Bahar,MD

Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran

Chronic myeloid leukemia was the first leukemia described and the first leukemia related with a consistent chromosomal aberation.
Stem cell transplantation was the first treatment modality that eradicated ph-positive clone and induced cure. The first report of this kind of treatment published more than 30 years ago in syngeneic twins and was then followed by HLA-mathched non-twin siblings and later in unrelated donors.
After production and dramatic results of (STI-571) and low toxicity profile of this oral medication stem cell transplantation for CML patients spent a phase of ambigiosity and criteria for selection of stem cell candidates was for a few years not clear.
Now we know that the best candidate for Stem Cell Transplantation are the patients who escape
the first molecular response and do not respond to second line drug therapy. In our country selection criteria for transplant candidates, regarding of low transplantation price and non insurance coverage of second line TK inhibitors are different. We have transplanted 256 CML patients in Shariati Hospital. It was the treatment of choice before 8-10 years ago.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Hematopoietic Stem Cell Transplantation in Lymphoma Patients: the Iranian Experience

Seied Asadollah Mousavi MD,Kamran Alimoghaddam MD, Mohammad Jahani MD, Babak Bahar MD,Amir Ali Hamidieh MD, Amir Kasaeian PhD , Ardeshir Ghavamzadeh MD.

Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran .Iran

Background:
Autologous hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment in patients with lymphoma including Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL).
Patients and Methods:
621 patients (385 male and 236 female) with a median age of 26 years (range: 10-78 years) in 331 HD patients and a median age of 33 years (range: 7-62 years) in 290 NHL Patients had received HSCT in our center from January, 1992 through January, 2015. The most common subtypes of NHL Patients were diffuse large B-cell lymphoma 42.5% . The most common status of disease before transplantation was First and second complete remission in NHL patients and second complete remission in HD patients. The sources of hematopoietic stem cells for lymphoma patients were 601 peripheral blood (278 NHL, 323 HD), 16 bone marrow (10 NHL, 6 HD) and 4 patients with combined Peripheral blood and bone marrow (2 NHL, 2 HD).
Results:
The median time (days) to Absolute Neutrophil Count >= 0.5 × 10^9/L was +16in HD and +11 in NHL patients. The median time (days) to platelet count >= 20×10^9/L was +24 in HD and +23 in NHL patients. The median follow up time was 76.7 months. The five years, disease-free survival (DFS) for HD and NHL patients was 66.62 and 60.86 respectively. The five years overall survival (OS) for HD and NHL patients was 80.27 and 72.73 % . Acute and chronic GVHD occurred in 11 (18.4%) and 9 (15.5%) in Allogeneic HSCT. The three years, DFS for autologous and allogeneic in NHL patients was 56.864% and 87.06% (p=0.000).The three years, OS for autologous and allogeneic in NHL patients was 68.68% and 89.66 %, (p=0.000).
Conclusion:
Our results confirm that HSCT is a suitable treatment in patients with NHL and relapsed HD. Both autologous and allogeneic HSCT are effective treatment options for relapsed and primary refractory disease.

Quality Management in Hematopoietic Stem Cell Transplantation

Christian Chabannon

Cell Therapy Facility. Institut Paoli-Calmettes. Comprehensive Cancer Centre & Aix-Marseille Université&Inserm CBT-1409. Centre de Recherches en Cancérologie de Marseille. France.

Quality management (QM) is an essential part of operations and services in many human activities such as the airline industry. Curiously, biomedical activities are quite slow to adopt similar rules, probably as the result of historical and cultural attitudes where the singular interaction of a physician with a patient is considered as an “artistic” and unique commitment rather than as the delivery of a global service. QM is an integral part of the manufacturing process for pharmaceuticals, medical devices or diagnostic kits by the industry, less so in the administration of medical care by hospitals, clinics and practitioners.
Hematopoietic stem cell transplantation (HSCT) is one setting where QM has started to be implemented. This came as the consequences of a few severe adverse events made public and the realization by professionals in the field that HSCT accumulates deviations from traditional schemes of medical care and drug delivery. FACT (Foundation for the Accreditation of Cell Therapy) in the USA and Canada, and JACIE (Joint Accreditation Committee for ISCT & EBMT) in Europe were the responses provided by continental professional societies in the field, with the goal to promote excellence and harmonization. The FACT-JACIE standards define requirements needed from the three major components of a transplant program – clinical, collection and processing - to achieve these goals. The 6th edition of the standards will be published in a few weeks, and incorporate further changes in medical practices as well as increased requirements for evaluation of clinical outcome.
Ten years after the accreditation of the 1st European transplant program, EBMT took advantage of its large-size registry to demonstrate an improvement in clinical outcome for patients transplanted in centers preparing for JACIE accreditation. A follow-up study confirmed the clinical benefit associated with JACIE accreditation. JACIE is now accrediting programs beyond European borders, including middle-east countries.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Allogeneic HSCT in Severe Acquired Aplastic Anemia

Jakob R Passweg

Hematology Division, Basel University Hospital, Switzerland

Allogeneic hematopoietic cell transplantation for marrow failure is a modern medical success story resulting in 70-90% long-term survival. First line treatment for acquired marrow failure includes immunosuppression with antithymocyte globulin and cyclosporine as well as bone marrow transplantation (BMT); decision algorithms are useful to determine appropriate approaches with younger patients and patients with more severe disease receiving BMT as first line treatment whereas other patients are treated with BMT after failing immunosuppression. Prospective and observational studies have determined standards for transplantation in patients with an available HLA identical sibling donor, including marrow as a stem cell source, cyclophosphamide with ATG for conditioning and cyclosporine and methotrexate for prophylaxis of graft versus host disease, and it is against these standards that future progress must be measured. In recent years, availability of well-matched unrelated donors has increased and results of unrelated donor transplantation are approaching those using matched sibling donors. In patients without a matched sibling or unrelated donor, alternative approaches, including cord blood transplants and transplants from haplotype mismatched donors, and relevant techniques, are discussed.

Outcome of Patients Activating an Unrelated Donor Search for Severe Acquired Aplastic Anemia

Andrea Bacigalupo,MD

Divisione Ematologia e Trapianto di Midollo, ** Divisione di Ematologia Clinica, IRCCS San Martino, Genova, *Cattedra di Ematologia and ***Statistica Medica ,Universita' Genova, Italy

Patients with severe aplastic anemia (SAA) without a sibling donor receiveimmunosuppressive treatment (IST) with anti-thymocyte globulin (ATG). In the caseof no response to IST, a voluntary unrelated donor (VUD) search is usuallystarted. This study analyzes the outcome of ATG-refractory SAA patientsactivating a VUD search. Of 179 patients, 68 had at least one HLA-A, -B, and -DR matched donor identified and underwent HSCT while 50 also with a donor were nottransplanted because of early death (8), late response to IST (34), transplantrefusal (1), or other (7). Conversely, 61 had no matched donor, 13 of thoseultimately received a mismatched HSCT. All but one received marrow stem cells.Among patients aged <17 years, those with at least one matched donor had asignificant higher 4-year survival as compared to others (79% ± 6% versus 53% ±10%, P=0.01). There was also a survival advantage independent of recipient age when the donor search was initiated in the recent 2000-2005 study-period (74% ±6% versus 47% ± 10%, P< 0.05). In multivariate analysis, the identification of amatched VUD tended to impact favourably on survival in patients with a recentdonor search (P = 0.07). This study provides evidence for the use of unrelateddonor HSCT in children and adults with IST-refractory SAA.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Cryopreservation of Hematopoietic Stem Cells for Therapeutic use, Strategies to Minimize Adverse Reaction

Mohsen nikbakht;SaeedMohammadi;ArdeshirGhavamzadeh;Kamran Alimoghadam;Zahra NooriKhameneh;RaziehPazahar;MehrnazShasti;FahimehRaofi

Research Institute for Hematology, Oncology and Stem Cell Transplantation,Theran University of Medical Sciences

Hematopoietic stem cell transplantation(HSCT) is the most effective therapeutic protocol which has been successfully developed for treatment of wide range of disorders, and cryopreservation of both autologous and allogeneic stem cell is increasingly being used to facilitate logistical challenges in coordinating the collection, processing, preparation, quality control testing and release of the final HSC product with delivery to the patient.
Direct infusion of cryopreserved cell products into patients has been associated with the development of adverse reactions, ranging from relatively mild symptoms to much more serious, life-threatening complications, including allergic/gastrointestinal/cardiovascular/neurological complications, renal/hepatic dysfunctions, and so on. In many cases, the cryoprotant agent used which is typically dimethyl sulfoxide (DMSO) is believed to be the main causal agent of these adverse reactions and thus many studies recommend depletion of DMSO before cell infusion.
We will try to explain the HSC cryopreservation, the side effects reported after transplantation, along with advances and applicable methods for reduction of DMSO concentration which helps to reduce the adverse reactions.

Cord Blood Banking in Iran National Cord Blood Bank

Mahin Nikougoftar Zarif.PhD*, Kamran Atarodi ,Mozhdeh Nakhlestani, Khadije Golzade, Nasim Ezzati, Somayye Ran balooch

***Corresponder Author Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran**

Introduction:Today umbilical cord blood (UCB) has known as a commonly used source of hematopoietic stem cells for allogeneic transplantation and many cord blood banks have been established around the world for collection and cryopreservation of cord blood units. Herein, we describe our experience at Iran National Cord Blood Bank (INCBB) during 4 years of activity.

Materials and methods:UCBs were collected from 5 hospitals in Tehran. All the collection, processing, testing, cryopreservation and storage procedures were done according to standard operation procedures. Total nucleated cells (TNC) count, viability test, CD34+ cell count, colony forming unit (CFU) assay, screening tests and HLA typing were done on all banked units.

Results:Within 6498 collected units, only 32.9% fulfilled banking criteria. The mean volume of units was 102.8±20.6 ml and after volume reduction the mean±SD of TNC, viability, CD34+ cells and CFUs was $10.36\pm3.28\times10^8$, 95.6±3.5%, $3.1\pm1.7\times10^6$ and $7.1\pm4.7\times10^5$, respectively. Nine units were transplanted in Shariati hospital.

Conclusion:In our country banking of UCB is new and high rate of hematopoietic stem cell transplants needs expanding CB banks capacity to find more matching units, optimization of methods and sharing experiences to improve biological characterization of units.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Post-HSCT Fertility in Patients Receiving non-TBI-Based Conditioning Regimen: A 23-year Experience in Iran

ArdeshirGhavamzadeh, Kamran Ali Moghaddam, MohamadVaezi, MohamadJahani, SeiedAsadollahMousavi, BabakBahar, HoseinKamranzadeh, Amir Ali Hamidieh, ZohreShahabi, AkramKarimi.

Hematology Oncology and Stem Cell Transplantation Research Center; Tehran University of Medical Sciences.

Introduction: Infertility is one of the late effects in patients receiving hematopoietic stem cell transplantation (HSCT).The aim of this study was to assess fertility in survivors following HSCT.
Material and Method: The study included 4407 HSCT survivors who underwent transplantation between July 3, 1990 and August 30, 2014. The inclusion criteria for women entered the study was the age of less than 50 years at the time of transplantation. HSCT recipients (females and males) aged over18 were determined eligible to participate in the study. We made contact with 2698 patients.
Results: The study group consisted of 1714(63.5%) males and 984(36.5%) females with mean age of 31.5 years (range: 2-78) at the time of HSCT. Median follow-up was 44 months (1-286).
In this study, pregnancies occurred in female HSCT recipients and in female partners of male recipients. There were 86 pregnancies (19 females and 67 males) following spontaneous conception (n=54) and in vitro fertilization (ivF) treatment with either their own eggs or donated eggs. The median age of pregnancy in our participants was 31 years. Autologous (n=34), allogeneic (n=50), and syngeneic (n=2) hematopoietic stem cells were primarily used in this study. AML (30%) and HD (16.3%) were common diseases among pregnant women. The interval time between HST and fertility was 63 months.
Conclusion: The results of the survey showed that some recipients are able to preserve their fertility following HSCT. In order to increase the rate of pregnancies in HSCT survivors, they should be informed about the impact of late effects of HSCT on their fertility prior to treatment entry.
Keywords: Bone Marrow Transplantation, Fertility, Late Effects

Skin Involvement in Chronic Graft-Versus-Host Disease

S. ZahraGhodsi MD,

Professor of Dermatology, Tehran University of Medical Sciences, Tehran, Iran

Chronic graft-versus-host disease (cGVHD) is a major complication of allogeneic hematopoietic cell transplantation (HCT).The pathogenesis of cGVHD is poorly understood. Incidence rates of cGVHD after allogeneic transplantation range from 6% to 80%. Symptoms usually present within 3 years after allogeneic HCT and are often preceded by a history of acute GVHD. Manifestations of cGVHD may be restricted to a single organ or tissue or may be widespread.

Diagnostic manifestations of skin involvement include *poikiloderma*, *lichen planuslikeeruption,deep sclerotic features*, *morphea-like* superficialsclerotic features, or *lichensclerosus-like lesions*. Severe sclerotic features characterized bythickened, tight, and fragile skin may be seen.A distinctive feature forcGVHD that is not sufficiently unique to beconsidered diagnostic ofcGVHD is depigmentation.

Dystrophy in nails consisting of longitudinal ridging, nailsplitting or brittleness, onycholysis, pterygium unguis, and nail loss are distinctive signs of cGVHD but are not sufficient for diagnosis.

Diagnostic features of oral chronic GVHD include*lichenplanus-like changes*,*hyperkeratoticplaques* (leukoplakia), or*decreased oral range of motion* in patients with sclerotic features of skin GVHD.

MildcGVHD may be treated eitherwith topical immunosuppressive(topical steroids, tacrolimus/pimecrolimus and phototherapy) agents or with systemicsteroidsalone.Treatment of moderate and severe cGVHDrequiressystemicimmunosuppression. Additional topical treatment maybe applied to speed up the response or to improve localresponse rates.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Hematopoietic Stem Cell Transplantation in Montasrieh Hospital of Mashhad University

Ghassemi A*, Bakhti O, Hamidi H, Ghahraman S

***Assistant Professor of Pediatric Hematology & Oncology, Mashhad University of Medical Sciences, Mashhad, Iran**

Hematopoietic stem cell transplantation have been used for the treatment and cure of a variety of cancers, immune diseases, metabolic and genetic abnormalities and hematologic disorders. Hematopoietic transplantation is a process to replace unhealthy bone marrow with healthy bone marrow.
First hematopoietic stem cell transplant (HSCT) have been performed at the Stem Cell Transplantation Center at Mashhad University of Medical Sciences 2 years ago.
We transplanted 24 patients in our center, mean age was 10 year.
There are 19(80%) cases that have received allogeneic HSCT and 5(20%) cases that have received autologous HSCT. Stem cell sources for patients underwent HSCT were bone marrow(1patient)and peripheral blood(23 patients).
We performed transplant in pediatric patients and mean age was 10 year. Hematopoietic Stem Cell Transplantation (HSCT) have been performed in wide variety of diseases such as AML(4), ALL(4),Non-NHL(2), thalassemia(2), Aplastic anemia(2), Fanconi anemia(2), Neuroblastoma(2), Sickel cell anemia(2), Willms tumor(1),Osteopetrosis(1), Hepatoblastoma(1) and Chronic granulomatous disease(1).
At present, 19 patients are alive and 5(20%) patients died and one patient with neuroblastoma had recurence in adrenal.
Hematopoietic stem cell transplantation is the choice of treatment for many malignant, nonmalignant and genetic diseases.

Hematopoietic Stem Cell Transplantation in Mahak (NGO)

HedayatiAsl A, Mehrvar A, Tashvigi M, Fallah V, Zangooei R, Olaad E, Faranoush M, Naderi A, Dinarooni P, Nejadbakhsh M, Najafi E, Ranjbar H

Department of Pediatrics Stem Cell Transplantation; Mahak Cancer Children's Hospital, Tehran, Iran

Objective: The Society to Support Children Suffering from Cancer, also known as MAHAK, was set up in 1991 as a non-governmental and non-profit organization. In the past two decades, the organization has attracted a vast public support and fulfilled a great part of its mission which is to support children with cancer, reduce the child mortality rate and create an appropriate environment that empowers families who have children with cancer. Pediatric Stem Cell Transplant also is used to treat many types of conditions affecting children and adolescent, including cancer and certain hematologic, immunologic and genetic disorders. The pediatric stem cell transplantation ward was inaugurated in Mahak hospital (Iran-Tehran) on April, 2012.

Pediatric stem cell transplant ward practice that performs aboutn30 transplants per year. All patients are kept in high-efficiency particulate air (HEPA) –filtered, positive-air-pressure –sealed rooms, and strict hand hygiene is practiced. A 14-bed transplant unit specially designed for the needs of patients undergoing a stem cell transplant. The first case was of a young girl suffering from ALL and transplanted from HLA-identical sibling.

Patients and Methods: We analyzed the outcome of 89 patients from a single institution who underwent allogeneic & autologous stem cell transplantation from between 2012 - 2014. Eighty one of patients had peripheral blood stem cell as the stem cell source, seven of patients' bone marrow and in 1 patient cord blood used. The majority of patients are: ALL=21, Neuroblastoma=17, AML=9, Hodgkin's dis=23 Retinoblastoma=4, Ewing's sarcoma=2, Rhabdomyosarcom=2, Wilm's tumor=2, Hepatoblastoma=1, Aplastic Anemia=2, Hemoglobinopathy=1, Germ cell tumor=3, Epedymoma=1, Osteopetrosis=1.The conditioning regimens used were mainly myeloablative in allogeneic transplantation. Age of patients 7 month to 26 years with median age 10 years, M/F = 56/33. Thirty-three patients transplanted Allo HSCT and 56 patients transplanted Auto HSCT. GVHD prophylaxis regimen was cyclosporine + Mtx in Allo HSCT. All patients engrafted. The type of donor in allogeneic SCT includes 28 related sibling and 5 unrelated allogeneic.

Results: In allogeneic PBSCT patients' median time to reach absolute neutrophil count (ANC) > 0.5 × 109/L was 11 days, and the median time to platelet count > 20 × 109 /L was 13 days vs 17and 21 days in Allo BM patients. In autologous PBSCT median time to reach absolute neutrophil count > 0.5 × 109/L was 12 days, and the median time to platelet count > 20 × 109 was 14 days (56 pt's). Acute graft-versus-host disease of grade II to IV was observed in 69% of patients and chronic graft-versus-host disease in 53% of patients. At present 80 pt's are alive and 9 pt's died due to VOD, hemorrhagic stroke and relapse.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

With a median follow-up of 18.5 months (2-30 months) after transplant the two years overall survival were 86.14% and event-free survival was 84.1%.In Hodgkin's disease patient's overall survival and event free survival after autologous SCT better than neuroblastoma. Conclusion: Autologous SCT can lead to durable remissions in children and adolescents with Hodgkin's disease & solid tumor. These results indicate that despite our ward is new status both allogeneic and autologous HSCT are feasible with outcomes similar to developed countries. These preliminary data suggest that HSCTs have been used as one of the standard treatments for hematological diseases and malignancies in Iran.

Experience of DKMS, German Bone Marrow Donor Centre

Gabi Rall, Alexander Schmidt

DKMS German Bone Marrow Donor Center, Tübingen, Germany

DKMS is the world's largest donor centre with globally more than 4,9 million donors. DKMS Germany was founded 1991 with now 3,7 Mio* donors. In 2004 DKMS Americas (now DBC US) was founded with now 580 000* donors, 2008 DKMS Poland with now 550 000* donors and last but not least in 2013 DBC UK with 78 000* donors. In 2014 we have recruited more than 1.000 000 donors worldwide.
Up to date we realized more than 46 000 stem cell collections.
Since years we focus to recruit young donors with recruitment drives in schools and universities.
We perform high resolution typing by NGS technology of 12 loci HLA A, B, C, DR, DQ and DP, mostly in our DKMS LSL. Since 2014 we have added blood group, Rh factor and CMV for those donors who are recruited with blood tests.
Beside the donor recruitment activity, donor retention and availability is most important. To keep the motivation of the donors high DKMS established several programs to contact donors regularly. Donor availability during confirmatory typing is 77,3%, 11,7% are TU, 3,9% are DD, 5,6% donors are NI. At workup stage 89,4% of the donors are available with6,5% TU, 1,3% DD and 1,5% NI. To further improve availability DKMS has started a program where donors with the most frequent European phenotypes are contacted and asked if they want to participate in this special program. Their typing profile will be updated and also a health questionnaire is checked. These donors have a very high commitment to donate. The availability at CT stage is >90% and 99% in the final stage. 40 of our donors donated for patients in Iran.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

"NGS and it impact on our Better Understanding of Immunogenetics in Hhealth and Disease"

NezihCereb, MD;

CEO & Co-founder.Histogenetics.NY ,USA

Next Generation sequencing (NGS) technologies has revolutionized the practice of current science and medicine by making various genetic information readily available. It became possible to understand the genetics basis of the complex diseases.
In my presentation I will share Histogenetics' experience in transition from high volume Sanger –based HLA typing to Illumina based HLA typing. Illumina based sequencing technologies along with the proprietary work flow process and informatics that we have developed at Histogenetics enabled us to perform higher resolution typing at lower cost and faster than Sanger technology. We are also able to provide ABO Rh genotyping along with HLA typing that could not have been possible with Sanger technology.
Beside the cost affective and highly parallel sequencing information that is obtain by Illumina technology PacBio SMRT sequencing technology made sequencing of long strands of DNA (up to 40 kb) possible for more accurate phasing and sequencing complex regions of the genome with accuracy that is not yet obtainable by other techniques.
For examples these developments enabled researchers to pinpoint the differences in diabetes associated DRB4 hapltotypes from healthy individuals with DRB4 haplotypes.
The future is poised to further these kinds of findings and change the practice of Medicine.

The Result of Pediatric Hematopoietic Stem Cell Transplantation in Inborn Errors of Metabolism Using Myeloablative Conditioning Regimen: Iranian Experience

M. Behfar, AA. Hamidieh, S. Basirpanah, A. Hosseini, A. Ghavamzadeh.

Hematology-Oncology and Stem Cell Transplantation Research Center/Tehran University of Medical Sciences,Tehran, Iran.

Aim: Inborn errors of metabolism (IEMs) are a progressive and multisystem disorder that usually are lethal in childhood. Hematopoietic stem cell transplantation (HSCT) has been established as an effective therapy for some type of IEMs.
Patients and method: In this retrospective study, 33 pediatric patients affected by IEMs, who had undergone HSCT in our center between 2007 and 2013 were enrolled. Twenty one patients were male. The median age at transplantation was 27months (range: 5monthars). Patients underwent transplantation from HLA-identical sibling donors (n=12), full matched other related donors (n=11), mismatched unrelated donor (n=4), matched unrelated donor (n=3) and HLA-haploidentical related donor (n=3). Peripheral blood (n= 14), bone marrow (n=14) and cord blood (n=5) were used as sources of stem cell. All patients received non TBI myeloablative conditioning regimen with Busulfan and Cyclophosphamide with or without Antithymocyte globulin. Cyclosporine with or without short course of methotrexate were used as Graft-versus-host disease (GvHD) prophylaxis regimen.
Result:Of 31 patient who were alive until 15 days after HSCT, Engraftment occurred in 29. During the follow up, two patients experienced secondary graft failure. At the present time, 22 patients with the median follow-up of 30 months (range: 12-63 months) are still alive and 20 of them (17 full chimerismand 3 mixed chimerism) have no evidence of disease progression. All eight patients who developed grade III-IV acute GvHD, had good response to therapy. Limited chronic GvHD was developed in only one patient. The most common causes of death were infection and disease progression.
Conclusion:As pre-transplant complication in IEMs patients, early transplantation using myeloablative conditioning regimen and HLA-matched related donors are suggested to improve patients' outcome.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Transplant-Associated Thrombotic Microangiopathy(TA-TMA) in Childhood

BibiShahin Shamsian,MD.

Pediatric Congenital Hematological Disorders Research Center. Mofid Children Hospital. Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Transplant-Associated Thrombotic Microangiopathy(TA-TMA)in Childhood is a multifactorial disease. it is associted with Hemolytic anemia- Microangiopathic,intravascular platelet activation, and formation of platelet-rich thrombi white clotwithin the microcirculation.In this disorder multiorgan involvement is seen.Transplant-Associated Thrombotic Microangiopathy(TA-TMA) in Childhood typically manifests as renal impairment,pulmonary hypertension, polyserositis, gastrointestinal symptoms and CNS injury.TA-TMA is seen in Allogeneic HSCT and much less frequent in the Autologous setting.TA-TMAs, occur a median of 150 days (mean 90 days) after transplant.The range of disease in TA-TMA patients might be mild, self-limited form to uncontrolled fulminant disease leading to death. TA-TMAs present diagnostic challenges because they may not clearly fall into one of the categories of the 2 major TMAs: atypical hemolytic uremic syndrome (A HUS) and thrombotic thrombocytopenic purpura (TTP).Also complications of the transplant itself, including Infection, GVHD, Disseminated intra vascuplar coagulation(DIC),Side effects of immunosuppressive drugs can mimic a TMA.

Pathogenesis:The pathogenesis in transplant-associated TMA is not very clear, it is believed that the disease process starts with Endothelialdamage.In this case, the abnormalities in vascular endothelium are independent from ADAMTS13 deficiency. TMA is a pathological definition and characterized by fibrinoid necrosis in vessel walls and arteriolar thrombus.Following intravascular thrombocyte activation due to microscopic damage, thrombus rich in thrombocytes develops in microcirculation.This process depletes thrombocytes , RBC are mechanically damaged due to microcirculation obstructed by fibrin particles or microthrombus.

Risk factors for TMAHSCT: Risk factors are conditioning regimens (busulfan, fludarabine, platinum-based chemotherapy), Infections ,GVHD and cytokines ,Calcineurin and mammalian target of rapamycin inhibitors (sirolimus),Coagulation cascade and endothelial markers , Female gender, Age: less frequent in children compared to adults, Extent of HLA mismatch, Severity of the primary disease, Use of ATG, Total body irradiation , Stem cell source(BMA, PB) and Complement

Clinical signs TA-TMAs: Mild hemolysis, severe Anemia, thrombocytopenia, fever, hematuria, mental disability, kidney failure requiring dialysis may be present in patients.LDH is increased, Haptoglobulin level is decreased. Indirect hyperbilirubinemia and

hemoglobinuria ,NRBC in peripheral circulation and fragmented erythrocyte ratio (4-10%)in transplant-associated TMA are seen.Thrombocyte consumption is increased although DIC is not present.Elevated LDH levels, hypertension, and proteinuria on routine urinalysis were the earliest markers of a TMA.Elevated serum C5b-9 levels with proteinuria were associated with very poor survival (<20% at 1 year).

Treatment ,TA-TMA: TA-TMA is difficult to diagnose and also difficult to treat. There is no any consensus on the therapy of TMA.

A work-up for infections should be instituted, Decrease or stop immunosuppressive therapy, Plasma Exchange has also been widely used for any form of TMA, including TA-TMA, for decades .The vast majority of patients with TA-TMA who are tested have ADAMTS13 activity levels above 5% to 10%TA-TMA, therefore should not be expected to respond significantly to plasma exchange unless an ADAMTS13-deficient state with activity of less than 5% to 10% is present, instances of which appear to be quite rare.

Rituximab , is of limited or no clinical value in the vast majority of patients with TA-TMA, who have ADAMTS13 activity levels >5% to 10%. Defibrotide is approve -Europe with antithrombotic and thrombolytic activity,inhibits TNF mediated endothelial cell apoptosis, anti-inflammatory and anti-ischemic effects.Main effect is local on vascular bed. (Daclizumab),Anti-CD25 antibody, TNFα inhibitors such as Etanercept and Infliximab.Ecluzumab (Soliris, Alexion, Anti-C5, available onlysince Sep 2011.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Peripheral Blood Progenitor Cell Collection for Childhood and Infancy Period

Julián Sevilla Navarro,MD

Hematología y Hemoterapia Hospital Infantil Universitario Niño Jesús.
Avenida Menéndez Pelayo, 65.28009-Madrid , Spain

Peripheral blood progenitor cells collection (PBPC) in children have largely replaced bone marrow as source of autologous stem cells partially due to their relatively easy collection. However, there is a concern regarding medical, psychological, social and technical difficulties in small patients. In our institution, we have performed this procedure during the last 20 years, with more than 200 apheresis for PBPC collection on small patients. H. Niño Jesús is a reference center for some hematopoietic stem cell transplant units for pediatrics along the country. We usually perform large volume leukapheresis (LVL), defined as the processing of more than three volumes of blood in a single session, in order to reduce adverse events related to the collection by decreasing the apheresis procedures. We performed most procedures with the Cobe Spectra Stem Cell Separator, although for the last years we have introduced in our regular practice the use of the Optia device.
Moreover, the use of children as hematopoietic stem cell donors represents for some colleagues an ethical dilemma. For some authors this is even more questionable if they underwent peripheral blood progenitor cell (PBPC) collection. We reviewed our experience on these procedures, reporting a low incidence of adverse events, and some variables that must be considered to improve the success of the collections.
In this presentation, the experience on this field will be reported by the author, with considerations regarding mobilization issues, technical aspects, and clinical variables related with adverse events and final product collected.

Outcome Improvement Following HaploidenticalStem Cell Transplantation in Patients with High Risk Leukemia: A Comparison of High Dose Post-transplant Cyclophosphamide (PT-CY) versus Prophylactic DLI

Kamran Alimoghaddam,[1]Ardeshir Ghavamzadeh,[1] Maryam barkhordar*[1]

[1]Tehran University of Medical Sciences, Hematology- Oncology and SCT Research Center, Tehran, Iran, Islamic Republic Of

Introduction:Haploidentical transplantation has become a clinical option for patients lacking an HLA-matched donor. Multiple methods have been made to improve outcome after haploidentical stem cell transplantation (SCT). High dose cyclophosphamide in the early post transplant period (PT-CY) is an effective strategy for GVHD prevention and engraftment facilitation due to immunogenic tolerance. Prophylactic DLI was applied to improve both relapse rate and immunity reconstitution after haploidentical SCT. Here we report the outcome of 40 patients of Haploidentical SCT in our center that randomizeded in two arm of PT-CY versus Prophylactic DLI.

Materials (or patients) and methods: From Jun 2010 to August 2014, 40 patients (27 male, 13 female) with high risk acute leukemia (29 AML, 11 ALL), whose have not suitable related or unrelated donor underwent haploidentical SCT from family members and randomized in two arms. Median patients age was 26.6 year (17-55 year) in PT-CY arm and27.3 year (13-58 year)in DLI arm. Identical regimen for conditioning and GVHD prophylaxis were used in two arms. The myeloablative conditioning regimen comprised busulfan and cyclophosphamide and ATG. GVHD prophylaxis consisted of cyclosporine and methotrexate. In PT-CY arm patients undergone HSCT and received additional dose of cyclophosphamide 50mg/kg at +3 and in DLI arm after transplantation patients received DLI from the same donor after subcutaneous G-CSF at day +30 with average cell dose 1-2×10^7 mononuclear cells (MNC)/kg.

Results: Totally 40 patients enrolled, 20 patients allocated in each arm. Median follow up was 720 days in PT-CY arm and 534 days in DLI arm. 5 patients in PT-CY arm and 7 in DLI arm were died. Cause of deaths were infection in 4 patients of DLI arm and 2 patients of PT-CY arm. diseaserelaps in 2 patients of PT-CY arm and 1 patient of DLI arm. Acute GVHD (grade 2-3) accur in 10 and 16 patient in PT-CY and DLI arm respectively (P-value=0.046).Chronic GVHD (moderate and severe) accur in 3 and 7 patient in PT-CY and DLI arm respectively (P-value=0.20). One year disease free survival was 79% and 84% in PT-CY and DLI arm respectively (P-value=0.97). One year overall survival was 76% and 71% in PT-CY and DLI arm respectively (P-value=0.43).

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Conclusion: The results of this study reveals no statistical difference between two arms in DFS and OS and chronic.GVHD, probably due to small size of patients and short time of follow up. However the acute GVHD was significantly lower in PT-CY arm.

Keywords: cyclophosphamide, DLI, Haploidentical transplantation

HCT for Bone Marrow Failure Syndromes

Marc Bierings,MD

Pediatric hematologist, Head, Dept of pediatric hematology and stem cell transplantation, Utrecht University Children's Hospital. Utrecht, The Netherlands.Also on behalf of R Admiraal, C Lindemans and JJ Boelens, Utrecht, The Netherlands.

(Pan)cytopenia in childhood can have various causes. Inherited marrow failure syndromes are rare, diverse and their phenotypes may vary. In case of persistent cytopenias underlying defects as in dyskeratosiscongenita, Shwachman Diamond and Fanconi anemia should be considered. Known etiologies are ribosomopathies, telomere length maintenance disorders and DNA breakage repair diseases.
These various syndromes may lead to an indication for HCT. Timing, conditioning regimen and donor/stem cell source choice should all be considered carefully by an experienced pediatric transplant team.
The Pediatric Diseases Working Party of the EBMT had a consensus meeting on the indications for HCT in these disorders in Vienna a few years ago. Results of this workshop will be discussed:
Diamond Blackfan anemia usually responds to steroid treatment and HCT is rarely needed. The phenotype can be difficult to separate from eg ADA2 deficiency, a recently identified disorder.
Severe congenital neutropenia (Kostmann syndrome) often responds to G-CSF treatment, persistent acquired G-CSF receptor mutations challenge transplant consideration.
In Fanconi anemia HCT results have improved dramatically after the introduction of fludarabin in the reduced intensity conditioning regimen, especially in the unrelated donor setting. Current discussions focus on the timing of transplant, the need to reconsider the role of irradiation in the conditioning and the development of comprehensive care teams for this complex chronic condition. In adult hematology there is an increased awareness that this disease may have its first presentation in adulthood with aplasia at a young age, familial myelodysplasia or leukemia, unusual malignancies or increased toxicity of chemo or radiation therapy.
This presentation will focus on the European consensus for HCT indications for these conditions and the results of HCT in a Dutch national cohort of Fanconi anemia of the past decades.
It is of great importance that cooperation between centres treating considerable numbers of these patients is explored, to increase the knowledge on genotypes and phenotypes and share experience in treating these vulnerable patients.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Neuropsychoimmunology of HSCT

Seyed Masoud Arzaghi

Seyed Masoud Arzaghi,MD

Psychiatrist, Fellowship in Psychosomatic Medicine,Assistant Professor.Elderly Health Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an aggressive, curative treatment for malignant and non-malignant hematologic conditions. The transplantation procedure is accompanied by significant physical, social and psychological stressors. This can lead to depression and anxiety. Most of the literature on allo-HSCT related complications focuses on the possible graft-versus-host disease (aGVHD), but many other symptoms observed that a cluster of distressing non-aGVHD related symptoms, such as fatigue, pain, poor appetite, sleep disturbance and importantly psychological distress, create considerable sickness during allo-HSCT affecting patients' daily functioning and necessitating the use of multidisciplinary care facilities. Even so, the mechanism underlying the development of multiple symptoms especially psychological dysregulations and neurocognitive dysfunctions during allo-HSCT has been proposed and it seems bidirectional relationship has been suggested underlying psychological problems and immunological dysfunctions. A great body of evidence proposes that cytokines play a role in sickness (for example, neuropathic pain, cachexia, chronic fatigue syndrome, disturbed sleep, and depression) in studies of both animal models and humans in cancer researchand in neuropsychoimmunology. For example dysregulations of inflammatory cytokines (eg, interleukin IL-1, IL-6, and tumor necrosis factor [TNF]-α) has been proposed as a predominant mechanism underlying sickness symptoms in patients with cancer. Along these lines, it can be argued that healthy brain function depends on the ability of the immune system to successfully regulate the production of neurotrophic factors. These include the secretion of growth factors, like BDNF and IGF-1, the preservation of homeostatic balance between pro- and anti-inflammatory cytokines within the CNS, and the efficient maintenance of the CNS cellular environment by appropriate clearance of cellular detritus and toxic compounds, such that normal neuronal development may occur through poor destruction or elimination of abnormal cells by reduced natural killer (NK) cell activity.
In this lecture, we aim to elucidate new horizons and suggesting some neuropsychoimmunological points of view to open new research field regarding bidirectional relation between HSCT and psychological status.

Human Pluripotent Stem Cell-Derived Hepatocyte-Like Cells Ameliorate Disease in the Carbon Tetrachloride-Induced Mouse Liver Injury Model

Hossein Baharvand[1,2], Ph.D.

Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, PO Box 19395-4644, Tehran, Iran.Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran.

In recent years, cell transplantation has drawn tremendous interest as a novel approach to maintaining or even restoring function of degenerative and trauma disease. Many researchers have sought to differentiate pluripotent stem cells into desired lineage cells including hepatocytes by translating the proof-of-principle concepts into more practical and feasible protocols for scale up and manufacturing of functional hepatocytes. Here, I describe a scalable stirred-suspension bioreactor culture of functional hepatocyte-like cells (HLCs) from the human pluripotent stem cells (hPSCs). The results have demonstrated that the generated hPSCs-HLCs showed functional HLCs characteristics, improved liver function, and extended the survival of carbon tetrachloride-treated mice while enriched cells based on one of their physiological functions, the uptake of acetylated LDL-DiI, infused into their spleens. Notably, no tumor formation was detected at 15 weeks post-transplantation. This integrated approach may facilitate biomedical applications of the hPSC-derived hepatocytes.

Comparing Intramyocardial Injection of Autologous Bone Marrow-Derived CD133(+) and Mononuclear Cells of Bone Marrow Cells and Coronary Artery Bypass Grafting for Acute Myocardial Infarction: A Phase II/III, Multicenter, Placebo-Controlled, Randomized, and Double-Blind Trial

MohammadHassanNaseri, MD, [a*]HodaMadani, MD, [b*]HosseinAhmadi, MD, [c] Maryam MoshkaniFarahani, MD, [d] DavoodKazemi-Saleh, MD, [e] HosseinHosseinnejad, MD, [f] SaeedHosseini, MD, [g]SepidehHekmat, MD, [h] ZarghamAhmadi, MD, [i]MajidDehghani, MD, [f]AlirezaSaadat,MD, [j]SouraMardpour, MSc, [b]Seyedeh-EsmatHosseini,BS,[b]MaryamEsmaeilzadeh, MD, [k]HakimehSadeghian, MD, [c]GholamrezaBahoush, MD, [l]AliBassi, MD,[m]Ahmad Amin, MD,[n]RoghayehFazeli, MD, [b]YaserSharafi, MD, [j]Leila Arab, MD, [b]MansourMovahhed, MD, [h]SaeidDavaran, MD, [c]NargesRamezanzadeh, BS, [c]AzamKouhkan, MD, [b]AliHazaveyi, MD, [o]MehrnazNamiri, MSc, [b]FahimehKashfi, MSc,[p] Ali Akhlaghi, MSc, [p]FattahSotoodehnejadnematalahi, PhD,[b]AhmadVosoughDizaji, MD, [q]HamidGourabi, PhD, [r]AbdolhoseinShahverdi, PhD, [b]HosseinBaharvand, PhD,[b]NasserAghdami, MD, PhD,[b,#]

*** These authors contributed equally to this work**

a. Department of Surgery, Baqiyatallah Hospital, Tehran, Iran.
b. Department of Regenerative Biomedicine at the Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
c. Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran.
d. Department of Echocardiography, Baqiyatallah Hospital, Tehran, Iran.
e. Department of Cardiology, Baqiyatallah Hospital, Tehran, Iran.
f. Department of Cardiac Surgery, Lavasani Hospital, Social Security Organization, Tehran, Iran.
g. Rajaei Cardiovascular Medical and Research Center, Tehran, Iran.
h. Department of Nuclear Medicine, Hasheminejad Hospital, Tehran University of Medical Sciences, Tehran, Iran.
i. Transplantation Research Center, NRITLD, MasihDaneshvari Hospital, ShaheedBeheshti University of Medical Science, Darabad, Niavaran, Tehran, Iran.
j. Department of Internal medicine, Baqiyatallah Hospital, Tehran, Iran.
k. Echocardiography Research Center, Rajaie Cardiovascular, Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
l. Department of Pediatrics, Aliasghar Children's Hospital, Tehran University of Medical Sciences, Tehran, Iran.
m. Department of Hematology and Oncology,Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran.
n. Department of Heart Failure and Transplantation, Fellowship in Heart Failure and Transplantation, Rajaei Cardiovascular, Medical and Research Center, Tehran University of Medical Sciences, Tehran, Iran.
o. Department of Internal medicine, Lavasani Hospital, Social Security Organization, Tehran, Iran.
p. Department of Epidemiology and Reproductive Health at the Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
q. Department of Reproductive Imaging at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.
r. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

 # Correspondence Address
Nasser Aghdami MD, PhD,
Royan Institute For stem Cell Biology and Technology, Akbari allay, Ejazi Ave, Zafaranieh St. Valiasr St, Tehran, Iran.
Tel: +98 21 22306485
Fax: +98 21 22310406
Email: Nasser.aghdami@royaninstitute.org

Short Title: Comparison of Intramyocardial Transplantation of Autologous Bone Marrow-derived Cells in AMI

Background: Meta-analyses of clinical trials suggest that intramyocardial administration of autologous bone marrow (BM)-derived CD133+ and mononuclear cells (MNC) may improve functional capacity and symptoms of patients with Acute Myocardial Infarction (AMI). However, outcomes of using different cell types have not been elucidated yet. This phase II/III trial is designed to determine whether autologous BM-derived CD133+ cells are as effective as BM-MNCs for the treatment of patients with AMI and for those undergoing coronary bypass surgery (CABG).
Methods: Patients (n=77) with AMI (anywhere from 10 days to 3 months post AMI) were enrolled in to six Iranian medical centers from January 2008 to July 2012. Candidates were randomly allocated into different groups, where one group (n=21) were treated with intramyocardial injection of 8 × 106 CD133+ cells, second (n=30) with 565 × 106 MNCs injection and placebo group (n=26) injected with normal saline and 2% autologous serum during CABG. Patients were assessed by stress echocardiography and single photon emission computed tomography (SPECT) six and eighteen months post to transplantation. Finally, data were compared with baseline and analyzed using MIXED model.
Results: The autologous transplantation of MNC and CD133+ cells improved cardiac function compared to placebo. These injected cells significantly increased ejection fraction 9% (P=0.01) and wall thickness 3.7 (P=0.03) determined by gated SPECT. In addition to this purified CD133+ cells significantly decreased the non-viable segmentsto1.5 (P=0.001) and 1.2 (P=0.01) in comparison to placebo and MNC groups, respectively.
Conclusions: The direct myocardial injection of autologous bone marrow cells in patients with recent MIundergoing CABG is feasible, safe and efficient. The CD133+ cells revealed a better functional outcome than MNC.
Keywords: Myocardial infarction, Cell therapy, Autologous transplantation, Bone marrow derived cells, Mononuclear cells, CD133+ cells

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Neural and Glial Cells Differentiation of Endometrial Stem Cells in Three Dimensional Scaffold

Jafar Ai[1,2],Somayeh Ebrahimi-Barough[1]

[1]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Brain and Spinal Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Neural tissue engineering is one of the most promising methods for treatment of nerve tissue injuries. Three-dimensional (3D) environment mimics in vivo conditions for cells. The cells 3D distribution and growth in the scaffold are both important for neural tissue engineering.
Materials and methods: Here we cultured endometrial stem cells in fibrin gel and evaluated cell viability after 8days and cell differentiation after 18days post culture. hEnSCs were isolated from donor tissue and were cultured in fibrin gel, then induced to neural and glial cells with growth factors (FGF2/EGF/PDGF-AA, RA) for 18 days. The viability of cells was analyzed by MTT assay for duration of 8 days cultured in fibrin matrix. Structure of fibrin matrix and cell morphology was analyzed with SEM, quantitative RT-PCR and immunohistochemistry were performed for neural and glial cells markers after cell differentiation in fibrin matrix.
Results: Results indicated that cell viability is enhanced in fibrin matrix after 8days and SEM observation have revealed that cells are in good integration with nano-fibers. However, immunocytochemistry and quantitative RT-PCR results for neural and glial cell differentiation markers showed that markers NF-L, MAP2, Olig2, Sox10, PDGFRa and A2B5 are expressed after 18days cultured in fibrin matrix.
Discussion: The results suggest that fibrin can provide a suitable three-dimensional scaffold for EnSCs differentiated cells for the regeneration of CNS.
Key word: endometrial stem cells, neural and glial cells, differentiation, neural tissue engineering, fibrin gel

Inhibition of HSP90 Makes Amniotic Epithelial Cells as a Potential Source for Cell Therapy of Cancer

Hassan Niknejad*[1], GhasemYazdanpanah[1], Masoumeh Mirmasoumi[1], Mahsa Khayat-Khoei[1].

[1]Department of Tissue Engineering, School of Advanced Technologies in Medicine, ShahidBeheshti University of Medical Sciences, Tehran, Iran.*Corresponding author:HassanNiknejad, PhD

Introduction:Human amniotic epithelial cells (hAECs) are the nearest cell layer of the amniotic membrane to the fetus which havestemnesscharacterestics. AECspossessimmunomodulatory and angio-regulating features which make them as suitable candidate for cancer therapy. In this study, we evaluated anti-cancer effects of hAECs.

Materials and Methods: Condition medium of the amniotic membrane or hAECs were added dose-dependently to the formerly cultured Hela or MDA-MB-231 cancer cell lines for an overnight. The viability of cancer cells was measured by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. The role of apoptosis in anti-cancer features of hAECs were assessed by terminal deoxynucleotidyltransferasedUTP nick end labeling assay (TUNEL assay) and immunohistochemicalmeasurement of caspase-3 and -8 expression. Effects of intact amniotic membrane (with AECs) and denuded AM (without AECs) on angiogenesis were analyzed using rat aortic ring assay.

Results:The supernatant of both amniotic membrane and hAECs reduced viability of both cancer cell lines in a dose dependent manner. The expression of caspase-3 and -8, as apoptotic markers, increased in the cancer cells treated with supernatants. Moreover, TUNEL assay revealed that viability of the cancer cells were decreased due to apoptosis. In rat aortic ring assay, hAECs inhibited angiogenesis on both mesenchymal and epithelial surfaces of amnion; however, removing of hAECslead to endothelial cell penetration and tube formation on the amniotic membrane surfaces.

Discussion: In this study, we showed that AECs have anti-tumor property owing to apoptosis induction and inhibition of angiogenesiswhich is possibly mediated by inhibition of heat shock protein 90. Further studies are needed to consider clinical anti-tumor applications of hAECs.

Key Words: Amniotic membrane, amniotic epithelial cells, apoptosis, angiogenesis, cancer cells

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Adipose Tissue Derived MesenchymalCells in Combination with Bone Marrow MNCs Improve Cell Therapy in Stroke

AlirezaShoae-Hassani[1], PhD; PeymanKeyhanvar[2,3], MD, PhD, Amir Ali Hamidieh[4],MD

[1]Applied Cell Sciences (Cell Therapy) department, School of Advanced Technologies in Medicine, TehranUniversity of Medical Sciences, Tehran, Iran.cell.therapy@yahoo.comTel. 09121454878
[2]Department of medical nanotechnology, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran.
[3]Rajaie cardiovascular research and medical center, Iran University of Medical Sciences, Tehran, Iran.
[4]Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Introduction: Stroke is a brain disorder that transforms patients permanently disabled. At thetime there are no known treatments for reversing this disorder. The use of autologous bone marrowderived mononuclear cells (MNCs) has been documented as treatment in such disorder; however, thistreatment has limited results. To overcome this limitation, we have used a combination of adipose derivedstem cell (ADSC) and MNCs in 4 doses, intrathecally, into adult rats.
Methods: Experimental model of cerebral ischemia have been developed in 12 adult rats (200± 20 g) tomimic human stroke. Group 1 (3 animals) was treated with 4 doses of MNCs (10^8 cells / dose), Group 2(3 animals) was treated with 4 doses of ADSC (10^8 cells/ dose). Group 3 (9 animals) was administered acombination of both cell types at each dose. All doses were administered intrathecally at a time interval offour weeks.
Results: Subjects were assessed for improvement in the muscle power, movement behavior and overallfunctional ability. It was observed that treatment with a combination cell therapy caused increase inmuscle power and improvement in behavior at a faster rate than that achieved by MNCs alone.
Conclusion: Our result is a preclinical evidence to prove that combination cell therapy with ADSCs &MNCs is safe and efficient in enhancing treatment rate. Of course extensive research will have to becarried out before this claim can be made as a technique for cell therapy.
Keywords: Adipose derived stem cell, Combinational Cell Therapy, Regenerative Medicine

Stem Cell; Some Challenges with Ethics and Legal Principles

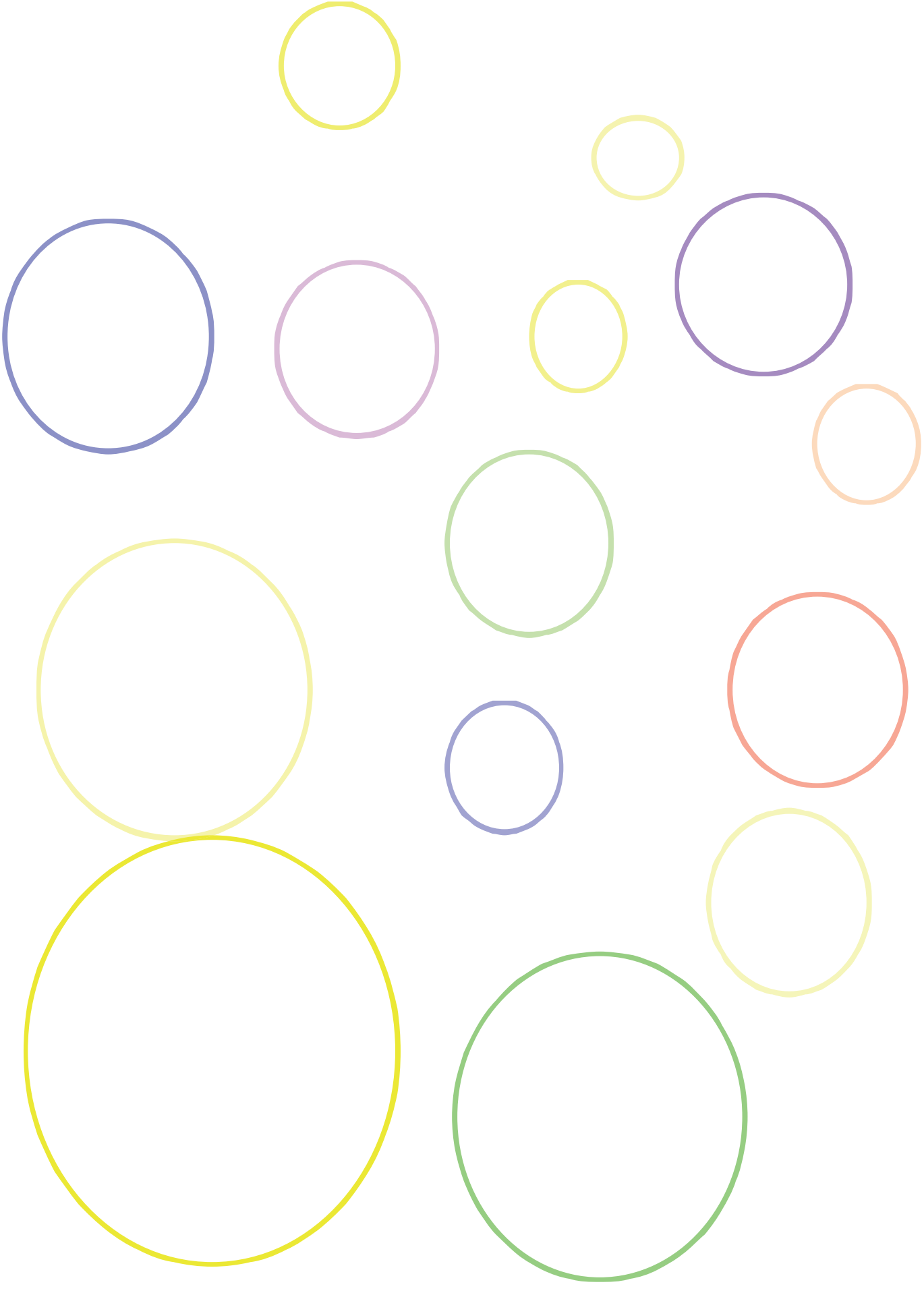
Arash Okazi

Department of Forensic Medicine, Tehran University of Medical Sciences

During recent decades, stem cell research has posed a challenge for politicians and national and international regulatory agencies. Despite challenges across different societies, stem cell Research continues to be conducted by researchers. The need for prevent unethical conduct and negative outcomes is recognized by many scientists. As a result, international and national committees have tried to guide stem cell activities ethically. General ethical guidelines such as the Belmont report, Helsinki declaration, and International Ethical Guidelines for Biomedical Research Involving Human Subjects should be observed by stem cell researchers. Establishment of international ethical guidelines and legal frameworks for human cloning was also considered at the end of the 20th century. The issue of reproductive cloning was discussed several times in the United Nations Educational, Scientific and Cultural Organization [UNESCO], the World Health Organization (WHO) and in different United Nations agencies. All of them believe that there are some important issues which may influence on this decision, such as: respect for human dignity, potential benefits for patients, safety of the process of research and therapy and informed consents of the participants.

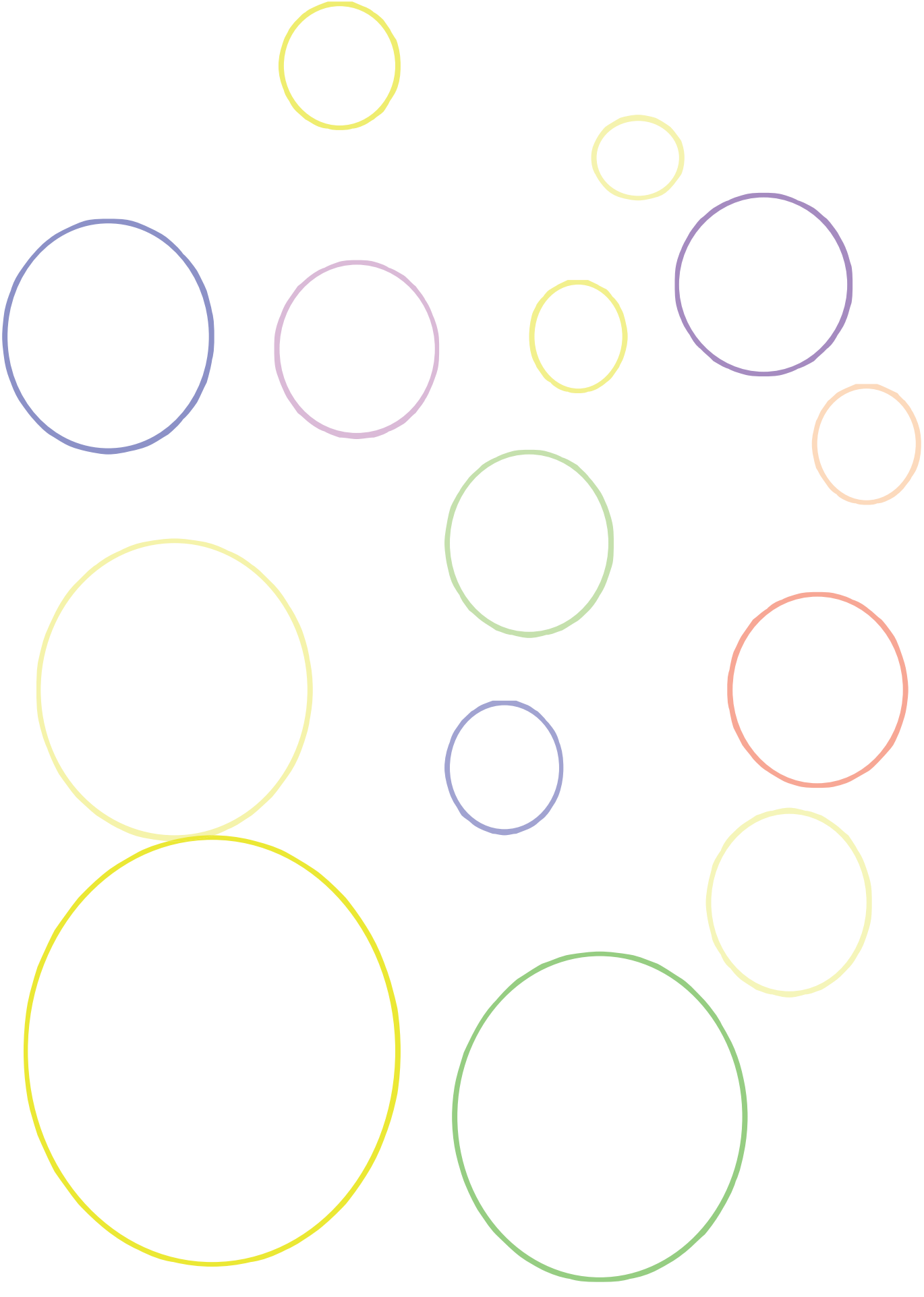
However Religions' perspectives and some variants like the moment when the soul arrives are other factors which affect on using stem cell. For example in Iran; the use of stem cells for therapy or scientific research is permitted as long as the cells' sources are permissible. For instance, the scholars in the conference of the Muslim World League's Islamic Jurisprudence Council held in Mecca in 2003 issued that: Adults who consent, placenta or umbilical cord blood, excess fertilized eggs produced during the course of IVF and spontaneously aborted embryos are some acceptable resources, and intentionally aborted fetuses are forbidden to be used as a source for stem cells.

It seems that rather than the universal ethical approved guide line for the stem cell research and therapy, each country needs a national rule for their activities which is coordinated with their religious belief.



The 1st International and 5th National Congress on Stem cells January 15-17, 2015; Tehran , Iran

Poster Peresentation



Clinical Standards of Good Practice in Advanced Therapy Medicinal Products Studies

Ramin Heshmat, MD, MPH, PhD- Head, Chronic Diseases Research Center (CDRC), Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences- Avicenna Clinical Research Group

Clinical trials, as the most advanced methodologies, provide proof for the efficacy, safety and adequate quality of novel drugs, treatments and medical interventions in order to achieve licensing and marketing. Conduction and documentation of such studies have to comply with strict regulations in order to allow their repeatability. These regulations and standards are summarized under the term “Good Clinical Practice” (GCP).
The GCP-regulations define all tasks, areas of responsibility, and procedures for the planning, authorization, application, and surveillance of trials, as well as their documentation and corresponding reporting. Actually, it is an international standard for the *design, conduct, performance, monitoring, auditing, recording, analyses*, and *reporting* of clinical trials which provides assurance that:

- the Data and Reported Results are Credible, and Accurate, and that is “Quality of Data”
- the Rights, Integrity, and Confidentiality of Trial Subjects are Protected, it means “Ethics”.

This guideline should be followed when generating clinical trial data that are intended to be submitted to regulatory authorities. Before medical products can be placed on the market, except for some special cases, they have been required to undergo an authorization procedure. This authorization is granted by Food and Drug Administration (FDA) in USA, and in Europe by the European Agency for the Evaluation of Medicinal Products (EMEA) and in Iran by FDO (Food and Drug Organization). Only after all proofs of the pharmaceutical or medical product quality, efficacy, and safety by non-clinical (laboratory/animal experiments) and clinical (probands/patients) studies have been provided according to the authorization standards, market-authorization for the medical product will be granted. Stem cells, stem cell therapy and regenerative medicine products are classified as “Advanced Therapy Medicinal Products = ATMPs” and their studies have to comply with the ATMP- regulation which became effective by EMEA in Europe and by Center for Biologics Evaluation and Research in FDA. Besides the clinical trials on ATMPs, to ensure the quality and safety of these products, the manufacturer of medicinal products has to comply with the international effective standards according to the rules of “Good Manufacturing Practice” (GMP). The corresponding EU-directive (Dir. 2003/94/EC) contains detailed rules and guidelines for quality management, certification, and documentation, regulates the equipment of the production facilities and the education of employees and administers issues about risk management.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The Principles of Cell Manufacturing for Clinical Applications

Hamid Reza Aghayan, Babak Arjmand, Ramin Heshmat

Chronic Diseases Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical sciences, Tehran, Iran

Cell based therapies provide exciting new opportunities to treat incurable diseases.Many therapies require large number of pure cell population, therefore the isolated cells need to be expanded and purified in vitrobefore transplantation. In vitro manipulation of cell products requires complex laboratory procedures that increasethe risk of possibly adverse events for the recipient.To minimize the associate risks of cell transplantation, adhering to current international standards for clinical grade cell manufacturing is critical. According to current European law and FDA regulations for phase 1 clinical trial, cell based products should be manufactured under principles of GMP. The main focus of this presentation will be on principals of Good Manufacturing Practice (GMP) which defines optimal quality and safety for cell based products.We also describe our experiences in clinical grade cell manufacturing. Among different elements, facility is the most obvious and tangible aspect of GMP. The controlled environment of a carefully designed, constructed, validated, and maintained cleanroom will minimize the risks of environmental contamination during aseptic processing and decrease the possibility of cross-contamination. Therefore we also discuss about different aspects of clean room facility with paying particular attention to facility design, qualification and maintenance.

Keywords: Cleanroom, Clinical grade, Cell therapy, GMP

Stem Cells Translational Medicine

Babak Arjmand[*1,2], Hamid-Reza Aghayan[1,2], Parisa Goodarzi[2], Fereshteh Mohamadi-Jahani[2]

[1]Chronic Diseases Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical sciences, Tehran, Iran
[2]Brain and Spinal Cord Injury Research Center, Tehran University of Medical sciences, Tehran, Iran

Stem cell therapy has introduced promising hopes for the treatment of various diseases. On the other hand, clinical utilization of stem cells needs to translate basic sciences and protocols before starting clinical phases by bridging stem cell research into clinical trials. Therefore stem cells translational medicine will open a new horizon in this area of research and practice. Accordingly, there are several risk factors relevant to safety issues of stem cell preparation and transplantation that must be considered in translational phase. For instance; transplantation site reactions, immune responses, biodistribution, ectopic grafting, unintended differentiation into another cell type, tumorigenicity, and lack of functional characteristics. In summary, to conduct clinical stem cell transplantation trials, the safety concerns must be carefully weighed against the potential benefits and all preclinical and clinical researches must be designed to elucidate potential safety concerns.
Key Words: Cell therapy, Safety concerns, Stem cells, Translational medicine

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The Role of Endometrial Mesenchymal Stem Cells in Neurodegenerative Diseases

Nasrin Lotfibakhshaiesh, MD, PhD
Assistant Professor, Department of Tissue Engineering

Large numbers of neurodegenerative disorders are all characterized by neuronal cell loss, associated with consequent loss of function and disabilities. The use of stem cells for neurodegenerative diseases has become of interest. Recent works in stem cell biology have opened up new insight in therapeutic strategies to replace lost cells with stem cells in injuries. Cell replacement therapy is a potential strategy for treating neurodegenerative diseases such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and multiple sclerosis and spinal cord injury. There is currently a great deal of interest in the use of MSCs to treat neurodegenerative diseases. Endometrial stem cells are a new source of stem cells present in the uterine endometrium that probably play a role in cyclic endometrial regeneration. This makes these cells a very interesting approach to studies in regenerative medicine, especially in cell replacement therapy. Many researchers showed the ability of endometrial stem cells to differentiate into multi lineage cells and including neuron-like cells, oligodendrocyte cells, adipocyte cells, Osteoblast cells. These cells can be characterized by the expression of specific gene markers such as CD146, CD90, and CD105.Much evidence suggests that EnSCs can be utilized in regenerative medicine. EnSCs can be used as immuno-modulatory agents to attenuate inflammation, are implicated in angiogenesis and vascularization during tissue regeneration, and can also be reprogrammed into induced pluripotent stem cells. Furthermore, EnSCs can be used in tissue engineering applications and there are several clinical trials currently in place to ascertain the therapeutic potential of EnSCs.Proposed regenerative approaches to neurological diseases using MSCs include cell therapies in which cells are delivered via intracerebral or intrathecal injection. MSCs transplanted into the brain have been demonstrated to promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons. Clinical trials for MSC injection into the CNS to treat traumatic brain injury and stroke are currently ongoing.

Evaluation of Supportive Capacity of Different Sources of Mesenchymal Stem Cells on Expansion of Umbilical Cord Blood CD34+ Cells

Kamran Atarodi Ph.D.
Dept. of National Cord Blood Bank. Iranian Blood Transfusion Org

Introduction: Allogenic transplantation with umbilical cord blood (UCB) is limited in adult recipient due to low CD34+ cell dose. Ex-vivo expansion of UCB is one potential solution to improve outcome and extend applicability of UCB transplantation. Simulation of the hematopoietic microenvironment nich is one of the acceptable methods for UCB CD34+ expansion. Nowadays many sources of mesenchymal stem cells (MSCs) are employed to expand CD34+ cells in co-culture manner. In this study the efficiency of MSCs from different source were evaluated.
Methods: Isolated CD34+ cells from UCB were co-cultured with extracted bone marrow (BM), placenta and umbilical cord (UC) MSCs for two weeks. In all culture conditions, stem cell factor, thrombopoietin and Flt-3 ligand as growth factor were used. At the end of each week, suitable aliquots of cultured cells were used to monitor total nucleated cells, CD34+ cells and colony forming cells (CFCs).
Results: The results showed that at the first week of expansion, there was statistically significant difference in supportive capacity of BM-MSCs on hematopoiesis and other sources (p value<0.05). But during the second week of co-culture, hematopoietic cell growth patterns have changed and UC-MSCs surpassed the competition from other sources. Compared to other sources, UC-MSCs lead to a higher expansion of CD34+ cells and induce more production of CFCs (p value<0.05)
Conclusion: It is concluded that UC-MSCs possess more potential application towards the generation of clinically significant cell number of umbilical cord hematopoietic stem cells with functional advantage than other sources for transplantation.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The Use of Stem Cells in the Treatment of Congenital Heart Disease in Children

Salehi Abarghuie F.[1] Moghrab M.[2] Riassi HR.[3]

[1]Assistant Professor of Pediatrics cardiology (MD), Faculty member of Medical School, Valie-e-Asr Hospital, Birjand University of Medical Sciences, Birjand, Iran
[2]Teacher, Faculty member of Nursing School,Birjand University of Medical Science,Birjand,Iran
[3]Neourlogist, Faculty member of Medical School, Valie-e-Asr hospital, Birjand University of Medical Sciences, Birjand, Iran

Today, According to the result of the use of stem cell therapy in all areas of medicine, this method is used in the treatment of congenital heart disease.
The dilated cardiomyopathy is most common heart disease with poor prognosis in children. It is often develops into an acute and chronic heart failure. It's occurred probably by viral & bacterial disease or unknown etiology or hereditary metabolic states. The goal of heart failure treatment is increase the chance of survival for to get to the heart transplantation time. In contrast to other congenital heart disease, neither it is a Palliative nor isn't it no curative method.
Key word: Congenital heart disease -stem cell
Method & matrials: No 45 patients were studied for a period of three months under echocardiography follow up, ECG & medical treatment in 92-93 that refferd to Heart Clinic Children's Hospital Medical Center.
Obtaining informed consent from them and were candidates for BMT.
The mean of age were 4±1.5 Yrs and in echo had EF= 36±5, LV MPI = 4.5±0.6, HR=130±5 beat/min, Mod to Sever MR, TR, Irrespectively.
Treatment of the heart failure were obtained in the patients with digoxin, captopril, furosemide, aspirin and warfarin if EF <30%.
After patient admission was done Holter monitoring and for patient's functional class was determine by an MPI isotope scan & cardiac stress test.
Bone marrow aspiration and cardiac biopsy were taken from all patients. All of samples of bone marrow stem cells were cultured, after 3-4 weeks sufficient volume of cultured stem cells were obtained ,at the second admission CBC, DIFF, BUN, Cr BS, Na, K, Pt, Ptt, INR, BG, RH, Troponin T, I were getting, an injection of the stem cells was done after healthy & safety other organs.
At The first by injection of contrast material in the aortic root was determined exact location of the coronary arteries. And then, the stem cells injected slowly through the Antegrade routed by 5-Fr R& LJD coronary catheters, in all patients were under Cardiac monitoring of myocardial ischemia and arrhythmias.

Results:

BMT	Arrhythmia	Ischemia & MI	Cure	Intervention	Weekly follow	Death
Case1	+		+Transiant	Lidocain	2-4-12	+5after mon
Case2	-	+	-	Transfer to CCU& Measurement troponin level		+
Case3	+		-	Lidocain,shock		+
Case4	-	-	+EF=37%_EF=45%	-	2-4-12	-
Case5	-	-	Stable	-	2-4-12	-
Case6	-	-	-	-	2-4-12	+3after mon
Result	2	1	2	3	2	4
Treatment			Continue & redue			cut

Discussion: In patients were poor Partial responses with serious complications, it is due to technical problems or lack of skills in using of this method, but BMT still seen as a modern therapeutic method with a clear horizon.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The Optimization of Isolating Hematopoietic Stem Cells of Umbilical Cord Blood

Mahdieh Ghiasi[1], Hoda Fazaeli[1], Mohsen Sheykh Hasan[1], Naser Kalhor[1*]

[1]Highly specialized jihad daneshgahi infertility treatment center, Qom branch, Iran
[*]Corresponding author

Introduction: Umbilical cord blood (UCB) banking has become a routine activity for providing hematopoietic progenitor cells for transplantation. The major problem with long-term cord blood (CB) banking is the required storage space. In this sense, many studies have been performed to establish techniques for volume reduction of CB units. Volume reduction which concentrates progenitor cells by reducing plasma and red blood cells is a widely used procedure in umbilical cord blood banking. This procedure must ensure high cell recovery, cell viability and RBC depletion by reducing the UCB units to a standard volume.
Material and methods: In this study we used the sedimentation agent hydroxyl ethyl starch (HES) and phosphate buffer saline (PBS) in different doses in order to find the best combination of these two material which leads to better cell recovery. Based on this we had three group with different doses of HES and PBS. On the other hand each group itself was divided into two sub groups based on the time which let the red blood cells (RBC) to settle.
Results: The obtained result showed that by increasing the HES dose the number of recovered cell enhanced. More over the comparison between rates of recovered cells in two groups which received equal doses of HES and PBS showed that giving longer time to blood unit for RBC settlement causes significantly better outcome ($p \leq 0.0$).
Discussion: From the time when UCB banks were established up to now, various different procedures have been described for volume reduction. These procedures are based on plasma and RBC depletion and the cell recovery is highly variable. In this study regarding the effect of HES on depletion of RBC it seems that the high dose of HES and giving more time can effectively enhance the rate of cell recovery.
Key words: Umbilical cord blood, Hydroxyl ethyl starch, Sedimentation

Neural Cells Differentiated from Adipose Derived Mesenchymal Stromal Cells Seeded onto a Cellular Spinal Cord Matrix Promotes Recovery of Spinal Cord Injury in the Rat Model

Rashin Mohseni [1], Amir Ali Hamidieh [2], Seyed-Abdolreza Mortazavi-Tabatabaei [1], Mahdi Tondar [3]

[1]Applied Cell Sciences (Cell Therapy) Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Biochemistry and Molecular & Cellular Biology, Georgetown University, United State.

Introduction: The cell based therapies are partially successful for the recovery of spinal cord injury (SCI). Recently, decellularized spinal cord scaffold which mimic native neurons environment have been prepared. Our study aimed to investigate whether the spinal cord lesion gap could be bridged by implantation of natural scaffold seeded with neural progenitor cell (NPC) derived from Adipose Derived Mesenchymal Stem cell (ADMSC) and their effects on functional improvement.
Methods: A laterally hemisected spinal cord injury lesion was performed in adult Sprague rats (n=16) and the acellular scaffolds (ASC) seeded with NPCs were implanted into the lesion immediately. Animals with hemisected SCI were divided into 3 groups after SCI: 1) animals treated by ASC scaffold implantation (n=4), 2) animals treated by the implantation of an ASC scaffold with ADMSCs (n=4), and 3) SCI only (n=4). Animals which executed laminectomy without SCI were considered as control (n=4). All rats' behavior was tested using the Basso–Beattie–Bresnahan (BBB) test twice a week for 10 weeks.
Results: Behavioral analysis showed that there was significant locomotor recovery improvement in treatment group as compared with the SCI only group ($p<0.01$). 5-Bromodeoxyuridine (Brdu)-labeled NPCs could also be observed in the implanted scaffold two weeks after implantation. Also host oligodendrocytes were able to migrate into the graft. Biotin–dextran–amine (BDA) tracing test demonstrated that myelinated axons successfully grow into the graft and subsequently promoted axonal regeneration.
Conclusion: This study revealed that decellularized spinal cord scaffold seeded with NPCs derived ADMSC is able to bridge a spinal cord gap and promote axon regeneration and functional recovery in rat's model of spinal cord injury.
Keywords: Adipose derived Mesenchymal Stromal cells, Spinal cord Injury, Neural cells

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Stem Cell and Gen Therapy for type 1 Ddiabetes from Murine Embryonic Stem Cells Using RNA Interferences

Seyed Ehsan Enderami[1], Masoud Soleimani[2] ,Seyedeh Elnaz Enderami[3], Reyhaneh Nasiri mansur[4]

[1]Ph.D Student of Medical Biotechnology, Zanjan University of Medical Sciences, Zanjan, Iran
[2]Associated professor of Hematology, Tarbiat Modares University, Tehran, Iran
[3]B.SC. Student of Biotechnology, Alzahra University,Tehran, Iran
[4]M.Sc. Student of clinical biochemistry, Zanjan University of Medical Sciences, Zanjan, Iran

Introduction: Embryonic stem cells (ESCs) are potential pluripotent cells derived from inner cell mass of embryonic blastocyst stage. So far, growth factors have been used for differentiation of ESCs to insulin producing cells. In the present study, in the absence of growth factors, siRNA was used to silence targeted genes.
Materials and Methods: In this study, embryoid bodies (EBs) were derived from murine ESCs. The EBs were then cultured in four groups; three test groups (containing culture medium with siRNA) and the control (the same culture medium used in test group without siRNA). After three weeks, differentiated cells were analyzed by using RT-PCR (expression of some pancreas-specific genes), immunocytochemistry (detection of insulin presence in cells) and ELISA (evaluation of the amount of secreted insulin to culture medium).
Results: The RT-PCR analysis of differentiated cells on three test groups showed expression of beta cell specific markers including insulin and Pdx1. The results of immunostaining showed that the insulin protein are expressed in differentiated cell of Foxo1 siRNA group and Foxo1/Gcg-siRNA group with different amounts and finally insulin secretion assay show that differentiated cells on Foxo1 siRNA group secreted more insulin in comparison with the other groups.
Discussion: Our data indicate that murine ESCs differentiate into insulin producing cell using siRNA, without growth factors. Therefore, siRNA can be used as a novel approach for generating insulin producing cells from ESCs in vitro.
Keywords: Differentiation,Embryonic stem cells, Insulin producing cells, siRNA

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The 1st International and 5th National
Congress on Stem cells
January 15-17, 2015; Tehran , Iran

Evaluation of Platelet Micro-Particles Effect on Level of CXCR4 Homing Marker Expression on Umbilical Cord Blood Derived CD133+ Cells

Moghaddam F.[1], Oodi A.[2], Nikougoftar Zarif M.[3], Amirizadeh N.[4]

[1]MSc of Hematology and Blood Banking, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
[2]PhD of Hematology and Blood Banking, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
[3]PhD of Hematology and Blood Banking, Assistant Professor, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.
[4]PhD of Hematology and Blood Banking, Associate professor, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

Introduction: Cord blood $CD133^+$ cells are able to maintain long-term hematopoiesis and differentiate to different hematopoietic lineages. CXCR4 over expression is involved in successful transplantation of hematopoietic stem cells in the bone marrow. Platelet micro-particles (PMP) contain CXCR4 markers and are able to transfer them into hematopoietic stem cells. Therefore, considering the importance of $CD133^+$ cells as primitive HSCs, effect of platelet micro particles on the expression levels of CXCR4 marker in these cells was examined.

Materials and Methods: Cord blood $CD133^+$ cells were isolated by MACS. Isolated cells were cultured into two groups one as control without adding anything and PMP prepared by freeze-thaw and sonication method at two different concentration with 5 and 10µg/ml were added to the other group. The cells were cultured for five days in stem span. Expression of CXCR4 surface marker was analyzed by flow cytometry, Fold change of total cell number and CFUs also were measured.

Results: Fold increase of $CD34^+$ cell percent (p=0.001), cells co-expressing CD34/CXCR4 (p=0.05), CFUs were significantly different between groups (p=0.001). The expression of CXCR4 surface markers in presence of 10µg/ml PMP concentration was significantly difference in comparison with control cells on the fifth day (p=0.05).

Discussion: In the present study it was observed that the exposure of $CD133^+$ cells isolated from cord blood to 10µg/ml PMP concentration not only interfere in cell proliferation but also increased the expression of CXCR4 surface marker significantly.

Key words: CD133 antigen, Stem cell, CXCR4 receptor, Platelet micro-particles.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Biochemical and Physiological Mechanisms are Involved in Chemotherapy Resistance of Cancer Stem Cells

Azadeh Anbarlou[1], Amir Atashi[1], Masoud Soleimani[1], Mahshid AkhavanRahnama[2]

[1]Department of Hematology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2]Department of Hematology, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction: Chemotherapy resistance is one of the important properties of cancer stem cells (CSCs).In cancer cells, this feature could be achieved by biochemical and/or physiological mechanisms. Biochemical resistance includes up regulation of genes such as ABCG2. ABCG2 is a member of ABC transporters which export toxic drugs to extracellular space. Moreover, weak distribution, hypoxia or acidic environment of tumor are the important factors which are involved in physiological resistance. Acidic extracellular tumor microenvironment caused by aerobic glycolysis phenomena (Warburg effect) that were seen in cancer cells.

Materials and methods: Jurkat T-leukemic cell line was cultured in RPMI-1640 medium and separated based on CD133, CSC marker, by immunomagnetic cell separation system and the purity ofCD133+ cells were evaluated by Flow cytometry. Also Jurkat cells were cultured in acidic pH (pH: 6.6) and normal pH (pH: 7.4) for 3 weeks.

Quantitative expression of ABCG2 gene in our groups was analyzed by qRT-PCR. To evaluate resistance of our groups against chemotherapy drug (doxorubicin), Annexin V- PI staining was performed and apoptosis in these cells was measured.

All tests were carried out in triplicate. Data means were compared using Student's t-test. Statistical analyses were performed by SPSS version 16.0 analytical software. Statistical significance was defined at $P< 0.05$.

Results: Quantitative gene expression analysis of ABCG2 by qRT-PCR showed that ABCG2 expression in CD133+ cells was 2.4 fold ($P<0.01$) in compare to CD133- cells. The level of ABCG2 expression in cultured cells in acidic medium was 6.2 fold ($P<0.001$) in compare to cultured cells in normal medium.

Annexin-PI staining of Jurkat cells showed that apoptosis in $CD133^+$ and $CD133^-$ cells was 36% and 60%, respectively. Moreover, Annexin-PI staining in cells cultured in acidic and normal medium showed that percentage of apoptotic cells was 9% and 25%, respectively.

Discussion: To evaluate biochemical and physiological mechanisms are involved in chemotherapy resistance of CSCs. Here, we showed that cells are cultured in acidic medium resulted in up-regulation of ABCG2 gene that is consistent with investigations on cells isolated tumors. Studies showed that acidic extracellular tumor microenvironment has critical role in response to chemotherapy drugs. Moreover, we demonstrated that expression of ABCG2 in $CD133^+$ is more than $CD133^-$ cells. The up-regulation of ABCG2 gene resulted in resistance of chemotherapy drug.

Altogether, we indicate that up-regulation of ABC transporters like ABCG2 is one of the important mechanisms in failure of chemotherapy protocols. Whether increased expression of these ABC transporters is caused by acidic extracellular tumor microenvironment or acidic extracellular tumor microenvironment lead to increasing these ABC transporters require further studies.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Adipose-Derived Stem Cells as a Feeder Layer Reduce P53 Gene Expression of Human Expanded Hematopoietic Stem Cells Derived from Cord Blood

Aisan Farhadi, Tahereh Foroutan

Department of animal science, faculty of biological science, Kharazmi University

Introduction: Insufficient numbers of hematopoietic stem cells (HSCs) in cord blood per unit have limited the use of this source in engraftment for treatment of diseases. Therefore, researchers use cytokines and feeder layers to enhance the proliferation of these cells. Feeder layers with secretionof cytokines and direct connections with HSCs can decrease the expression of cell cycle inhibitor genes and increase self-renewal of HSCs. In this research we investigate the expression of p53, a tumor suppressor gene, in human expanded CD34+ stem cells derived from cord blood after co-culture with adipose-derived stem cells (ADSCs) as a feeder layer.

Materials and methods:CD34+ cells were cultured for 7days with cytokines such as Stem cell factor (SCF), FLt3-Ligand (FL), Thrombopoietin (TPO) , and ADSCs feeder layer including: (a) directly in contact with a feeder layer (b) separated by a transwell insert membrane (c) without a feeder layer. Gene expression was evaluated by real-time reverse transcriptase-PCR.

Results:The expression of p53 in the expanded CD34+stem cells on ADSCs feeder layer was the lower than the other groups. The low expression of p53 indicates the increase in self-renewal of HSCs. Therefore, the proliferation of CD34+ that cultured on a feeder layer was higher than the other groups.

Discussion: Direct cell-to-cell contact between CD34+ cellsand a feeder layer is necessary for HSCs expansion. It has been reported that highly dynamic culture events occurred in HSC-MSC co-cultures, involving cell–cell interactions, which preceded HSC expansion. Direct cell–cell contact is necessary for the supportive effect of the feeder layers. In this study, we observed that the expression of p53 in CD34+cells cultured directly on ADSCs feeder layer was lower than the other groups.

Key words: adipose-derived stem cells, feeder layer, P53, Hematopoietic stem cell

Induction of Human Umbilical Wharton's Jelly-Derived Mesenchymal Stem Cells towardMotor neuron-like cells

Zohreh Bagher[1], Somayeh Ebrahimi-Barough[2,3], Mahmoud Azami[2], Hamid Mirzadeh[4],Mansooreh Soleimani[5], Jafar Ai[2,3], Mohammad Reza Nourani[6], Mohammad Taghi Joghataei[7,1]

[1]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Iran University of Medical Sciences,Tehran, Iran
[2]Department of Tissue Engineering, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences,Tehran, Iran
[3]Brain and Spinal Injury Research Center, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
[4]Faculty of Polymer Engineering, Amirkabir University of Technology, Tehran, Iran
[5]Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[6]Genomics Division, Chemical Injury Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[7]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran.

Corresponding author: Mohammad TaghiJoghataei, Ph.D; Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran. E-mail: joghataei@iums.ac.ir

The most important property of stem cells from different sources is the capacities to differentiate into various cells and tissue types. However, problems including contamination, normal karyotype and ethical issue cause many limitations in obtaining and using these cells from different sources. The cells in Wharton's jelly region of umbilical cord represent a pool source of primitive cells with properties of MSCs. The aim of this study is to determine the potential of human Wharton's jelly-derived mesenchymal stem cells (WJMSCs) for differentiation to motor neuron cells. WJMSCs were induced to differentiate into motor neuron- like cells by using different signaling mlecules and neurotrophic factors in vitro. Differentiated neurons were then characterized for expression of motor neuron markers including Nestin, PAX6, NF-H, Islet1, HB9 and Chat by quantitative reverse transcription PCR and immunocytochemistry. Our results showed that differentiated WJMSCs could significantly highly express motor neuron biomarkers in RNA and protein levels after 15days. These results suggest that WJMSCs could be programmed to motor neuron-like cells and might provide a potential source for cell therapy in neurodegenerative disease.
Key words: Human Umbilical Wharton's Jelly-Derived MSCs, Motor Neuron, Differentiation

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Beta Adrenergic Receptors Agonist may Facilitate Mobilization of Hematopoietic Stem Cells to Bloodstream

Fakhredin Saba[1], Masoud Soleimnai[1]*Majid mokhtari[1], Fatemeh Sayyadipoor[2]

[1]Department of Hematology, School of medical science, Tarbiat Modares University, Tehran Iran
[2]Biotechnology Department, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Corresponder: Masoud soleimani: soleim_m@Modares.ac.ir

Introduction: CXCR4/CXCL12 axis dynamically mediates hematopoietic stem cell trafficking in the bone marrow (BM). Granulocyte colony stimulating factor (G-CSF) as the most effective mobilizing agents induces CXCL12 secretion from BM stromal cells into circulation. However, the effect of G-CSF on CXCR4 expression of HSC remains elusive. The nervous system regulates HSC migration through neurotransmitter of receptors. Beta adrenergic receptors as one of the neural receptors are expression by HSC. Here, we examined the effects of isoproterenol as agonist of beta on CXCR4 mRNA levels of cord blood hematopoietic stem cell.

Materials and methods: CD34+ cells were isolated with FACS from human cord blood and were cultured for 1 to 5 hours in the presence of beta adrenergic receptors agonist (isoproterenol) (10μM) and G-CSF(100nM). CXCR4 mRNA was measured by real time pcr. Results were expressed as mean ± standard deviation of the mean (SD). Statistical analysis was performed using SPSS 19.

Results: The results showed that isoproterenol would significantly increase expression of CXCR4 approximately 4-fold after 1 hour incubation at 37oc. However, Continuous CXCR4 stimulation with beta agonist leads to reduce its expression. Furthermore, our results showed indicated that G-CSF had no direct effect on CXCR4 expression of HSCs in vitro, but, co-stimulation G-CSF (100 nmol/ml) and isoproterenol (10μmol/ml) resulted in a dramatic increase in CXCR4 levels (12- and 6-fold increase after 1 and 3-hour incubation), illustrating immediate activity of catecholamine and G-CSF in expression of CXCR4/SDF1 axis.

Discussion: Today, circulating HSCs associated with G-CSF injection are used as the main cells source for BM transplant in various diseases such as leukemia. However, sometimes despite appropriated doses of G-CSF injection, insufficient HSCs would release into circulation known poor mobilization that effect on outcome and therapeutic process patients. Hence, to optimize HSCs collection, the identification of mechanisms and strategies involved in HSCs egression from BM is considered important. According to the proposed model, released norepinephrine controls G-CSF-induced up transient up-regulation of

CXCR4 in the bone marrow leading to hematopoietic stem and progenitor cells (HSPCs) mobilization. Our study has broadened the understanding of the function of the sympathetic nervous system in mobilization, demonstrating that bone marrow innervation regulates the retention HSCs through its effects on CXCR4 and SDF-1. On the basis of our results, we propose that myeloid cytokines such as G-CSF have indirect functions in the regulation of human CD34+ cells. Consequently, these cytokines would induce adrenergic signals by up-regulating the expression of neuronal receptors on hematopoietic progenitor cells, thus augmenting their response to neurotransmitters, leading to enhanced mobilization of HSC to the circulation.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Expression and Purification of Chimeric Protein Containing Human Prostate Stem Cell Antigen and Heat Shock Protein-70

A. Ghamari*[1,2], M. Ebrahimi[3,4], E. Hosseini[3,5]

[1]Division of Human Genetics, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Cellular And Molecular Biology Department, Faculty Of Life Sciences, Tehran North Branch, Islamic Azad University, Tehran, Iran
[3]Department of Bacterial Vaccine and Antigen Production, PasteurInstitute of Iran, Tehran, Iran
[4]Cellular And Molecular Biology Department, Faculty Of Life Sciences, Boushehr Branch, Islamic Azad University, Boushehr, Iran
[5]Azad university of Damghan, Damghan, Iran

Introduction:Prostate cancer is the most common cancer with a high mortality rate in males. Prostate stem cell antigen (PSCA), is a surface glycoprotein and has a 35% homology with stem cell antigen-2. PSCA is expressed in 86% of prostate cancer specimens with high tissue specificity. Studies have confirmed that vaccination based on PSCA enhances the cellular and humoral immune responses and inhibits the growth of PSCA-expressing tumors. So, PSCA may be a potential target for prostate cancer immunotherapy. Heat shock protein-70 (HSP70) is a major molecular chaperone, which assists in transport, assembly and folding of proteins in the cytoplasm transmembrane. Previous studies have demonstrated that vaccination with HSP70-peptide complexes elicit specific antitumor responses. These findings suggest that HSP70 is involved in the process of antigen presentation and has potential as animmune-adjuvant chaperone for specific antigens in vaccines.
Materials and methods: The desire recombinant gene based on PSCA and HSP70 which designed and analyzed by bioinformatics software were chemically synthesized. pET28a were used as an expression vector for transformation of competent BL21(DE3) Escherichia coli. The expression of chimeric multitope in recombinant bacteria induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG). Nickel affinity chromatography were used for purification of chimeric protein. The purified chimeric protein were identified and analyzed by SDS-PAGE and western blotting.
Result:The chimeric gene can clone in prokaryotic system. The expression of proteins corresponding to the predicted size were induced in the presence of IPTG. Recombinant fusion proteins were purified by Nickel affinity chromatography. Identification of recombinant

fusion protein were performed by SDS-PAGE and western blotting that confirmed the presence of the chimeric protein.
Discussion:In this study, we presented evidence that human HSP70 enhances the solubility of PSCA. The chimeric multitope were successfully expressed to a high level in E. coliin soluble form and it is convenient for purification. Following three steps of purification, a purity of greater than 95% of the recombinant fusion proteins was obtained. Western blotting revealed that the recombinant fusion proteins obtained via purification had the same immunological characteristics.In conclusion, the present study confirmed the potency of human HSP70 as a molecular immune-adjuvant chaperone and PSCA for a recombinant protein vaccine, which lays the foundation for the development of vaccines for prostate cancer and further clinical research.
Key words: PSCA, HSP70, Prostate cancer, Chimeric protein, Vaccine

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

In Silico Analysis of Chimeric Protein Containing Human Prostate Stem Cell Antigen and Heat Shock Protein-70

A. Ghamari*[1,2], M. Ebrahimi[3,4], E. Hosseini[3,5]

[1]Division of Human Genetics, Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Cellular And Molecular Biology Department, Faculty Of Life Sciences, Tehran North Branch, Islamic Azad University, Tehran, Iran
[3]Department of Bacterial Vaccine and Antigen Production, PasteurInstitute of Iran, Tehran, Iran
[4]Cellular And Molecular Biology Department, Faculty Of Life Sciences, Boushehr Branch, Islamic Azad University, Boushehr, Iran
[5]Azad university of Damghan, Damghan, Iran

Introduction: Prostate cancer is the most common cancer with a high mortality rate in males. Prostate stem cell antigen (PSCA), is a surface glycoprotein. PSCA is expressed in 86% of prostate cancer specimens with high tissue specificity. Studies have confirmed that vaccination based on PSCA enhances the cellular and humoral immune responses and inhibits the growth of PSCA-expressing tumors. So, PSCA may be a potential target for prostate cancer immunotherapy. Heat shock protein-70 (HSP70) is a major molecular chaperone, which assists in transport, assembly and folding of proteins in the cytoplasm transmembrane. Previous studies have demonstrated that vaccination with HSP70-peptide complexes elicit specific antitumor responses. These findings suggest that HSP70 is involved in the process of antigen presentation and has potential as animmune-adjuvant chaperone for specific antigens in vaccines.
Materials and methods:In the present study, we successfully constructed recombinant gene producing chimeric protein based on PSCA and HSP70 which lays the foundation for the development of a vaccine for prostate cancer.
We have designed an immunogencomplexeconsist of PSCA with overall length genes of HSP70 that represents a three-dimensional epitope of chimeric multitope protein. The construct were analyzed bybioinformatic'ssoftwares. Stability, proper energy level, linear and discontinuous B-cell epitopes, MHC class I and II binding peptides of chimeric protein were predicted.
Result: The designed chimeric moltitope had stability, proper energy level and same immunogenicity as the original protein's epitopes. The chimeric gene can clone in prokaryotic system.Our data indicates that epitopes of the synthetic chimeric protein could induce both B-cell and T-cell mediated immune responses which are important for a protective vaccine against prostatecancer.
Discussion: Studies have identified a low-expression of PSCA in normal prostate and a high-expression in prostate, bladder and pancreatic cancer. Further studies demonstrated

that the antibody of PSCA inhibits the growth of prostate cancer. The function of HSP70 in immune-adjuvant therapy has been identified and many studies have confirmed that vaccination with HSP70-peptide complexes and HSP70-antigen fusion proteins reconstituted in vitro with genetic recombination elicit antitumor immune responses. The present study confirmed the potency of human HSP70 as a molecular chaperone to use as immune-adjuvant for this recombinant protein. Our data may also suggest this synthetic chimeric protein as a vaccine candidate subunit against prostate cancer. Much work is needed to be performed to establish this notion which is the theme of our future research.

Key words: PSCA, HSP70, Prostate cancer, Chimeric protein, Vaccine

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Streptomyces Gqinglanensis Crude Eextract Can Induce Early Chondrogenesis of Mesenchymal Stem Cell

Rezvan Rezazadeh[1,2], Javad hamedi[1,2*], Ehsan Arefian[1,3], Fatemeh Mohammadipanah[1,2], Reza Ardeshiri[3], Zahra Borughani[1,2]

[1]Department of Microbial Biotechnology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran
[2]Microbial Technology and Products Research Center, University of Tehran, Tehran, Iran
[3]Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran

***Corresponding author: jhamedi@ut.ac.ir**

Introduction: Joint pain is one of the major causes of disability in the community that may be a result of trauma, tumor ablation or degenerative diseases such as Osteoarthritis and substantially decreases quality of life. Due to avascular nature, cartilage tissue doesn't have access to the body's regeneration system and has a very low capacity for regeneration. Until now, numerous biological approaches for articular cartilage repair have been developed, but they are in experimental phase and their efficacy is questionable. On the other hand, *Streptomyces* are one of the most valuable bacteria, can produce diverse biological and medical compounds.
in the present study, we wished to investigate whether the secondary metabolite of *Streptomyces* can launch chondrocyte differentiation procedure in adipose tissue derived stem cell (ADSC).
Methods: *Streptomyces qinglanensis* 172205(UTMC1116) was obtained from UTMC (University of Tehran Microorganisms Collection). Mentioned strain was grown in ISP2 culture in seed medium for 24 hours. After that fermentation was done in ISP2 culture at 28° C and 220 rpm for 7 days. Culture filtrates were obtained after centrifugation and the supernatant was extracted with equal volume of ethyl acetate at room temperature for 1h with vigorous shaking. After drying, extracts storage at -20°C. To investigate the effect of secondary metabolites of *Streptomyces* on the ADSC, cells at passage 3 were cultured in DMEM, supplemented with 12% fetal bovine serum, in a humidified atmosphere at 37°C with 5% CO_2. The ADSC viability was determined by MTT test after 48 hours. To measure gene expression of SOX9, ADSC were treated with 4 μg/ml microbial crude extract for 7, 14 and 21 days. The cells were then lysed in RNX-Plus (Low Copy RNA Isolation)-reagent. After which the total RNA was isolated and cDNA was synthesized, real time-PCR was performed according to the manufacturer's protocols. To examine extracellular matrix, Alcian blue was done on third week after treatment.
Results: In the present study, we evaluated the ability of Streptomyces qinglanensis 172205 crude extract to induce chondrocyte differentiation. According to the MTT assay, the crude

extract of related strain with the concentration of 0.5-4 μg/ml didn't interfere cell viability. Real time-PCR was used to study SOX9 gene expression as an early marker of chondrogenesis at 7, 14 and 21 days after treatment. Gene expression analysis was performed using REST software. Following the treatment for 7 days of culture, SOX9 gene expression didn't significantly increase in comparison to control group (p=0.340). On 14 days after treatment, the expression of SOX9 show dramatically increase by mean factor of 12.381($p \leq 0.00$). In contrast, expression level of SOX9 didn't significantly increase in comparison to control group on third week after treatment (p=1.00). It should be noted that, Alcian blue staining also showed much more intense in the sample group compared to the un-stimulated group.

Discussion: *Streptomyces* are the most biotechnologically valuable prokaryotes. They have ability to produce about half of the discovered bioactive secondary metabolites, notably antibiotics, antitumor agents and enzymes. The present study suggest that *Streptomyces qinglanensis* 172205(UTMC1116) metabolites can act as potential candidate for stimulating of chondrocyte differentiation.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Secondary Metabolites of *Streptomyces Shaanxiensis* Stimulate Neuron Differentiation in Mesenchymal Stem Cells

Rezvan Rezazadeh[1,2], Javad hamedi[1,2*], Ehsan Arefian[1,3], Fatemeh Mohammadipanah[1,2], Zahra Borughani [1,2]

[1]Department of Microbial Biotechnology, School of Biology and Center of Excellence in Phylogeny of Living Organisms, College of Science, University of Tehran, Tehran, Iran
[2]Microbial Technology and Products Research Center, University of Tehran, Tehran, Iran
[3]Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran
***Corresponding author: jhamedi@ut.ac.ir**

Introduction: Adipose tissue derived stem cells(ADSC) have been demonstrated to be able to differentiate into mesenchymal and non mesenchymal lineage, including cells with characteristics of neuronal tissue. On the other hand, the regeneration process in the human adult nervous system is complicated and loss of neurons is thought to be irreversible. This inability to generate replacement cells brings great challenges to researchers and a large number of publications focused on this area. According to these reasons, increasing number of various protocols have been reported to induce stem cell differentiation relied on variety bioactive molecules include growth factors and chemical supplements, such as β-mercaptoethaol, dimethylsulfoxide, butylated hydroxyanisole, retinoic acid, isobutyl methyl xanthine, dibutyryl cyclic AMP, etc. *Streptomyces* are one of the known prolific sources of bioactive molecules with a range of biological activities that may act as pharmaceutically useful compounds.
In the present study we extended these observations to test the hypothesis that secondary metabolites of *Streptomyces* can induce ADSC neuronal differentiation by exposure to crude extract of *Streptomyces*.
Methods: The *Streptomyces shaanxiensis* CCNWHQ 0031 designated UTMC 540 was obtained from University of Tehran microorganisms collection (UTMC). Fermentation of related strain was done in ISP2 culture and incubated at 28 ^{o}C, 220 rpm, 168 h. The secondary metabolites were extracted with equal volume of ethyl acetate at room temperature. After drying, crude extract was preserved at -20°C. To investigate the effect of microbial crude extract on ADSC, cells at passage 3 were maintained in DMEM, supplemented with 12% fetal bovine serum, in a humidified atmosphere at 37°C with 5% CO_2. Toxic effect of microbial metabolites were tested on ADSC by using MTT assay after 48 hours. According to the obtained result of MTT test, ADSC were treated with microbial extract for 7, 14 and 21 days in 12 well plate. In order to examined gene expression, total RNA was extracted and after cDNA synthesis, RT-PCR and Real-time PCR were performed and the results were monitored using Rotor gene Q-Real time analyzer. In this study, the

housekeeping gene β-actin was used as internal control. Real-time PCR statistical analyses were carried out using Rest software.
Results: In the present study, the crude extract of *Streptomyces shaanxiensis* CCNWHQ 0031 was selected as treatment to study neuronal differentiation. MTT assay showed that secondary metabolites of *Streptomyces shaanxiensis* CCNWHQ 0031 with the concentrations of 0.5-4 μg/ml could not interfere ADSC viability. Real time PCR was used to study nestin expression as neuronal marker. Following the treatment for 7 days of culture, nestin gene expression was decreased toward unstimulated ADSC group($p=0.020$). On day 14, the expression of nesin is down- regulated ($p\leq0.00$). In contrast, quantitative analysis of nestin show dramatically increase after third week ($p\leq0.00$).
Discussion: Preliminary studies have demonstrated that *Streptomyces* have an extraordinary ability to produce diverse pharmaceutical secondary metabolites. This investigation suggest that *Streptomyces shaanxiensis* CCNWHQ 0031 metabolites can act as potential candidate for neuronal stem cell differentiation.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Cultured Allogeneic Fibroblasts Injection in Comparison with Cultured Fibroblast on Amniotic Membrane Scaffold in Treatment of Dystrophic Epidermolysis Bullosa; A Pilot Clinical Trial

Moravvej H, MD, Naseh MH, MD, Piravar Z, PhD

Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Epidermolysis bullosa (EB) is a blistering disorder, caused by as much as 14 variable gene mutations . Recessive dystrophic epidermolysis bullosa (RDEB) is a distinct subtype of EB caused by different types of mutation in type VII collagen gen (COL7A1). It has also a wide range of severity based on the complete absence or decreased amount of type VII collagen (C7) at the dermoepidermal junction (DEJ).
In the absence of a definitive treatment, different modalities including gene therapy, protein replacement, and cell-based approaches have been used to treat the wounds of RDEB. Cell-based treatment is one of the promising methods of wound healing. Fibroblasts, as the producer of collagen, have a special place in both gene and cell-based approaches to the treatment. Molecularly engineered RDEB fibroblast that overexpress human C7 can home at lesions in murine skin when injected intravenously and promote wound healing by producing C7 and forming anchoring fibrils at DEJ. (4). Amniotic membrane alone was used successfully in dressing and promoting closure of EB wounds (5); however, we could not find any work regarding fibroblasts on amnion scaffold used in treatment of EB wounds. Different methods using fibroblasts have been examined to treat recessive dystrophic epidermolysis bullosa
Aim: to compare the intradermal cultured allogeneic fibroblast injection and Fibroblast seeded on acellular amniotic membrane scaffold in healing these wounds.
Methods: seven patients (four females and three male) were recruited and seven wounds were assessed in each patient (a total of 49 wounds) : three wounds were treated with intradermal fibroblast injection, three with fibroblast on amniotic membrane scaffold, and one was just dressed with Vaseline gauze as control for others. The changes in wounds size was assessed by photos and a software after two weeks, after 12 weeks, 6 month and, one year after treatment. QWS was used to assess the severity off wounds. In addition, a biopsy and antigen mapping were performed to determine the presence or absence of collagen type 7 in dermoepidermal junction.
Results: In both treated area the QWS was significantly decreased ($P<0.0001$). The wound size was significantly smaller after two weeks in those treated with fibroblast injection in contrast to fibroblast on amniotic membrane scaffold ($P<0.0001$). After second week, no significant changes in wound sizes was seen.

Conclusion: the injection of fibroblast results in significantly better wound healing in comparison to fibroblast on amniotic membrane.

Key words: epidermolysis bullosa; fibroblast; amnion membrane; intralesional injection

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Cord Blood Hematopoietic Stem Cell Expansion in Fibronectin Coated Micro Cavities

Mohamad Hossein Moghadasi[1], Masoud Soliemani[2], Abbas HajifathAli[3], Ali Akbar Movasaghpour Akbari[4]

[1]Labafi Nedjad Hospital ,Social society organization of IRAN,Tehran-Iran
[2]Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[3]Department of Hematology, Taleghani Hospital, Shahid Beheshti Medical University, Tehran, Iran
[4]Department of Hematology and Oncology, Tabriz University of Medical Sciences Hematology and Oncology Research Center, Iran

Introduction: The use of ex vivo-expanded cord blood hematopoietic stem cell (CB-HSC) for hematopoietic stem cell transplantation continues to increase .However; sufficient ex vivo expansion of undifferentiated and proliferating HSCs for transplantation is limited. These cells have a low rate of engraftment to bone marrow. In this study, the influence of microstructure and fibronectin interactions on HSC expansion were investigated.
Materials and Methods: It prepared a set of fibronectin-coated micro cavities. Umbilical cord blood (UCB) derived HSCs were cultured in the fibronectin coated micro cavities in presence of efficient of exogenous cytokines. TCPS-based cultures were performed in parallel as controls. On day 12, CD133 was determined using FACS. Colony-forming units (CFU) activity was evaluated
Results: When UCB-derived CD133+ cells were cultured in the fibronectin coated micro cavities system for 12 days .It observed an increase in the number of total nucleated cells (65fold) and CFU (1.2-fold). The number of CD133+ cells increased 6.5fold.
Discussion: This results show that ex vivo culture of CB- hematopoietic stem cells in fibronectin coated micro cavities is associated with increase the expansion rate. Fibronectin coated micro cavities provides an ex vivo mimicry of bone marrow niche Therefore, this strategy could be useful for HSC expansion.
Keywords: hematopoietic stem cells – Cord blood – Fibronectin Coated Micro Cavities

Effect of Low-Intensity Pulsed Ultrasound on Chondrogenic Differentiation of Human Adipose Stem Cells and Bone Marrow Stem Cells

Hajar Shafaei [1,2], Marco N. Helder [3], Behrouz Zandieh-Doulabi [2], Peter A. Nolte [4], Jenneke Klein-Nulend [2]

[1]Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam, University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands
[3]Department of Orthopaedic Surgery, VU University Medical Center, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands
[4]Department of Orthopaedic Surgery, Spaarne Hospital Hoofddorp, Hoofddorp, The Netherlands

Objective: New approaches in cartilage regeneration focus on the use of stem cells combined with a chondrogenic scaffold, mechanical loading, and growth factors. Adipose tissue is an easily accessible and abundant source of adipose stem cells (ASC), when compared to bone marrow stem cells (BMSC). Transforming growth factor-β (TGFβ) is known to induce chondrogenic differentiation of mesenchymal stem cells (MSCs), but several disadvantages are associated with its use such as the formation of an unfavourable hypertrophic cartilage phenotype followed by endochondral ossification instead of permanent chondrogenesis. Low-intensity pulsed ultrasound (LIPUS), a form of micromechanical stimulation, also induces chondrogenic differentiation of MSCs, but whether it has similar disadvantages as caused by TGFβ treatment is unknown. Moreover stem cells obtained from different sources might respond differently to chondrogenic inducers. The aim of this study was to investigate whether or not LIPUS stimulates stable cartilage formation in ASC and BMSC without hypertrophy. ASC and BMSC monolayer and micromass cultures were established, whereby only micromass cultures provide a 3D structure resembling the condensation of mesenchymal stem cells, i.e. the first step of cartilage formation in vivo. The effects of LIPUS on cell morphology and gene expression of chondrogenic markers specific for permanent cartilage, as well as gene expression of chondro-osteogenic markers specific for transient cartilage were determined in ASC and BMSC in the presence or absence of TGFβ.
Methods: Human ASC and BMSC monolayer and micromass cultures were treated with or without LIPUS (30 mW/cm2, 20 min/day) and with or without TGFβ (10 ng/ml) for 4 or 14 days. Alcian blue staining of ASC and BMSC monolayer cultures and BMSC micromass cultures was performed to show proteoglycan formation at day 14. Chondrogenic gene expression of Sox9, aggrecan (AGG), collagen type 2B (Col2B), cartilage oligomeric matrix protein (COMP), and Link protein, as well as chondro-osteogenic gene expression of runt-related transcription factor-2 (Runx2), alkaline phosphatase (ALP), collagen type 10 (Col10),

collagen type I (Col1), and matrix metalloproteinase-13 (MMP13) was assessed in ASC and BMSC monolayer and micromass cultures at day 4 and 14 by real time RT-PCR.
Results: Alcian blue stainingof monolayer cultures revealed similar proteoglycan formation in LIPUS-treated ASC as well as in TGFβ-treated ASC. All BMSC micromass cultures, either without or without LIPUS or TGFβ, showed enhanced chondrogenic differentiation compared to BMSC monolayer cultures. Cell aggregates, indicative for cell condensation and transformation of fibroblastic cells into round cells, were only observed in ASC monolayer cultures but not in BMSC monolayer cultures. In ASC micromass cultures, LIPUS stimulated chondrogenic gene expression of Sox9 (1.4 fold), AGG (1.4 fold), and COMP (1.5 fold) at day 14. In both ASC and BMSC micromass cultures, LIPUSdownregulated the expression of Runx2, ALP, Col10, Col1 and MMP13 compared to TGFβ-treated cultures at day 4 and 14. After 14 days, the effect of LIPUS and TGFβ on chondrogenic differentiation of ASC micromass cultures as assessed by chondrogenic gene expression were similar.
Conclusion: Our results suggest that LIPUS, similar as TGFβ, effectively stimulates chondrogenic differentiation of ASC and BMSC in vitro. LIPUS might be an excellent option to replace TGFβ treatment for the induction of chondrogenesis in ASC and in BMSC, albeit to a lesser extent, since it causes a decrease in hypertrophic gene expression. Finally, ASC might provide a feasible, cheap, and efficient tool for application in cartilage tissue engineering compared to BMSC.
Keywords: Adipose stem cells; Bone marrow stem cells; Chondrogenesis; Cartilage tissue engineering; Low-intensity pulsed ultrasound; Transforming growth factor-β

Generation of Functional Retinal Pigmented Eepithelium in Vitro

Hamid Aboutaleb kadkhodaeian [1,2], Taki Tiraihi[*1], Hamid Ahmadieh[3], Hosein ziaii Ardakani [1,3], Narsis Daftarian[3].

[1]Shefa Neuroscience Research Center, Khatam-Al-Anbia Hospital, Tehran, Iran
[2]Dept. of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, P.O. Box 14155-4838, Tehran, Iran.
[3]Department of Ophthalmology Labbafinejad Medical Center Pasdaran Ave. Boostan 9 St. Tehran, 16666, Iran.

***Correspondence: Taki Tiraihi, PhD, Professor of Department of Anatomy, Tarbiat Modares University, Tehran, Iran. Tel: +98-21-88790109 Fax: +98-21-88678104.**

Introduction: Retinal pigmented epithelium (RPE) have a broad spectrum of function in vivo such as phagocytosis of POS. Therefore loss of this layer could effect on vision in disease such as age related macular degeneration. Production of cells that have function in vitro is the first step to try treatment of disease related to the sensory retina and RPE layer.
Materials and methods: Bone marrow stromal stem cells extracted from male sprago-dawly rats and after cultivation, differentiated to neural stem cells. then NSCs again differentiated to retinal pigmented epithelium and after 7-14 days, phagocytosis ability of them investigated with internalization of POS labeled with fluorescent dye from same retina.
Results: Results showed that NSCs could differentiated to retinal pigment epithelium that phagocytosis of POS and internalize it in vitro.
Conclusion: In our investigation for the first time, differentiation of NSCs to RPE cells well done in vitro. Moreover this cells could phagocyte and internalized POS in vitro that is very important for cell therapy in degenerative disease such as age related macular degeneration and retinitis pigmentosa.
Keywords: BMSCs, NSCs, RPE, POS phagocytosis.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Photoreceptor Cell Derived from Bone Marrow Stromal Stem Cells in Vitro

Hamid Aboutaleb kadkhodaeian [1,2], Taki Tiraihi[*1], Hamid Ahmadieh[3], Hosein ziaii Ardakani [1,3], Narsis Daftarian[3].

[1]Shefa neuroscience research center ,Khatam-Al-Anbia Hospital, Tehran, Iran
[2]Dept. of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, P.O. Box 14155-4838, Tehran, Iran.
[3]Department of Ophthalmology Labbafinejad Medical Center Pasdaran Ave. Boostan 9 St. Tehran, 16666, Iran.

Introduction: A hereditary degenerative disease of the retina, retinitis pigmentosa (RP), is the leading cause of visual handicap among working populations in developed countries, with an estimated 1.5 million patients worldwide. Here we used simple method for production of photoreceptor cells in vitro.
Materials and methods: Bone marrow stromal stem cells derived from male pigmented rats. The cells differentiated under the specific medium. After 14 days in vitro the specific markers for sensory retinal cells investigated to determine the differentiation of BMSCs to retinal cells.
Results: Result showed that after 14 days in vitro BMSCs cells differentiated to cells similar to sensory retina in morphology manner. The specific markers for each cell revealed differentiation of BMSCs to retinal cells in vitro.
Conclusion: For the first time in our study, we could differentiate the BMSCs cells to retinal cells such as photoreceptor, amacrine, ganglionic and muller glial cell in a simple method.
Keywords: Photoreceptor cell, Retinal cells, BMSCs, Differentiation.
*Correspondence: Taki Tiraihi, PhD, Professor of Department of Anatomy, Tarbiat Modares University, Tehran, Iran. Tel: +98-21-88790109 Fax: +98-21-88678104.

The Differentiation of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells into Hepatocyte-Like Cells Expressing Antithrombin III

Zeynab Saie[1], Amir Atashi[1], Masoud Soleimani[1]

[1]Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Introduction: Antithrombin III (ATIII) is a serine protease inhibitor (SERPIN) that physiologically inactivates many enzymes in the coagulation cascade. It is produced mainly in the liver. ATIII deficiency ,an autosomal dominant disorder, is a risk factor for venous thromboembolism. Anticoagulants such as heparin, fondaparinux, enoxaparin and AT concentrates are current treatment options for individuals with ATIII deficiency , but nowadays stem cells have received particular attention as an attractive therapeutic tool for cell therapy. Mesenchymal Stem Cells (MSCs) possess the ability to produce functional hepatocyte for clinical applications. Due to easy isolation and expansion in culture with low tumorigenesity and teratoma formation MSCs become an attractive candidate for use in regenerative medicine. In this study, MSCs were used to produce hepatocyte- like cells and the gene expression of ATIII in differentiated cells was measured.
Materials and Methods: MSCs were isolated and cultured from umbilical cord blood and the surface phenotype of these cells was characterized via flowcytometry. UCB-MSCs were exposed to different liver-specific factors (hepatocyte growth factor, oncostatin M, Insulin-Transferrin-Selenium, dexamethasone, and nicotinamide) for 21 days. Total RNA was extracted from differentiated cells and also from untreated MSCs, as a control. Then cDNA was generated and hepatic differentiation was assessed via RT-PCR for liver-specific genes, including albumin, alpha-fetoprotein, HNF4a, CK18, CK19and for ATIII as a coagulation-related gene.
Result: After 21 days of induction cells with round and spindle-shaped morphology, which is characteristic of hepatocytes, was observed . In addition, successful differentiation into hepatocyte-like cells was determined by detecting expression of liver-specific genes such as albumin and alpha-fetoprotein. These differentiated cells also expressed ATIII .
Conclusion: UCB-MSCs exhibit the potential to differentiate into hepatocyte-like cells expressing ATIII in vitro.There is not much information about using MSCs for the treatment of liver disease, especially coagulation factor deficiency such as ATIII deficiency. Hence UCB-MSCs may serve as an ideal source for cell therapy of coagulation factor deficiencies.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Adipocyte Derived Stromal Cells (ADSCs) Application to Promote Epidermal Regeneration

Seyed Abdolreza Mortazavi-Tabatabaei, MD, PhD (Presenter)
Alireza Shoae-Hassani PhD

Applied Cell Sciences Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Mesenchymal-Epithelial interaction plays an essential role in organogenesis and tissue regeneration at both embryonic and adult stages. Wound healing is a promising model for studying the mechanisms of tissue regeneration of various organs. This healing process demonstrates dynamic regenerative processes consisting of inflammation, angiogenesis, tissue remodeling and scarring. In wound healing of the skin, epidermal regeneration is a critical event for reorganizing normal cutaneous structure. We have shown that a mesenchymal cell type of mature adipocytes promotes the reorganization of the epidermal layer together with keratinocyte growth and differentiation.
Materials and methods: Keratinocytes were obtained from the epidermis separated from the dermis. They were cultured and maintained in a complete medium of Ham's F-12 medium supplemented with 15% FBS and 50µg/ml an aminoglycoside antibiotic. To analyze the effects of the mesenchymal cells on the epidermal regeneration by keratinocytes, we reconstructed skin in vitro by coculture of keratinocytes with ADSCs. In addition, to decide whether mesenchymal-epithelial interaction was mediated by a direct cell-cell contact, keratinocytes and mesenchymal cells were cocultured under a separate condition. The culture assembly was fixed with 5% formalin, routinely processed, and then vertically embedded in paraffin. Deparaffinized sections were stained with H&E. Cell proliferation was examined by immunohistochemistry with mouse monoclonal proliferating cell nuclear antigen (PCNA) antibody. To estimate the differentiating properties of keratinocytes, monoclonal cytokeratin 10 (CK 10, a marker of suprabasal keratinocytes) and CK 14 (a marker of basal cells) antibodies were used in immunocytochemistry.
Results: ADSCs clearly promoted the stratification of keratinocytes, resulting in the formation of an epidermal layer consisting of basal, granular and cornified layers. We have shown that ADSCs promote epidermal regeneration in a skin reconstruction model. Keratinocytes without mesenchymal support disappear in a culture assembly after 14d. This suggests that mesenchymal support is critical for the survival, growth, and differentiation of keratinocytes.
Discussion: Keratinocytes with ADSCs as well as keratinocytes in vivo expressed CK10 in suprabasal layer, but not in basal layer. However, CK14 was displayed in both basal and suprabasal keratinocytes of regenerative epidermis with mesenchymal support, whereas it is expressed only in basal cells in vivo. Mesenchymal-epithelial cross-talk in skin is controlled

by various molecules, including IL-1, c-Jun, JunB, and KGF. Probably KGF protein was expressed in ADSCs under their direct contact with keratinocytes, suggesting that ADSCs may be involved in KGF production during epidermal regeneration. This suggests that ADSCs in that order may be involved in the mesenchymal-epithelial cross-talk in skin. We have shown that direct contact between keratinocytes and ADSCs is required for the skin-specific morphogenesis. This suggests that ADSCs may be applicable to cellular therapy for skin injuries.

Key Words: Adipocyte, Stromal Cells, Epidermal, Regeneration

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Ex Vivo Expansion of Cord Blood Mononuclear Cells by Umbilical Cord Blood Platelet Lysate

Monireh Mohammad [1, 2], Masoumeh Nouri[1], Marzieh Ebrahimi[1, 3]

[1]Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.
[2]Payame Noor University. Taft. Iran
[3]Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

Introduction: Platelets are blood cells that assist homeostasis. These cells have granules Containing calcium ions, serotonin and fibrinogen, CSF, IGF, EGF, IL8, IL-1β, FGF-1, TGF-β, PDGF, VEGF and b-FGF. Platelet extract has been introduced as a supplement for mesenchymal stem cell culture in cell therapy. The purpose of this study was to investigate the effect of Umbilical Cord Blood Platelet Lysate (UCB-PL), in proliferation, survival and differentiation rate of mononuclear cells derived from cord blood.
Materials and Methods: Platelet Lysate were prepared by mechanical method in concentration rang 2 ×10^9plt / ml from cord blood. Mononuclear cells were isolated by using hydroxyethyl starch from cord blood. They were seeded in 5 × 10^5-1× 10^6celles/ml and cultured in presence of FLT3 (50ng / ml), Tpo (10ng / ml), SCF (50ng / ml) concentrations of UCB-PL (0-10%)
In the positive control group, cells were cultured in a medium containing cytokines while negative controls were cultured in a medium lacking cytokines. The effective dose and cell viability were assayed by Trypanblue. Colony Assay method was used to assess the differentiation of mononuclear cells. The expression of $CD34^+$ as a stem cell marker was performed by QRT- PCR method.
Results: Higher concentrations of 5% UCB-PL have shown cytotoxic effects. In the presence of 5% UCB-PL, MNCs had higher proliferation than other groups. In this particular group significant increase of colony forming units Granulocytes - Erythroid - Macrophages - Megakaryocyte (CFU-GMMM) and granulocyte - macrophage (CFU-GM) were detected. An increased expression of CD34 + gene in cultured MNCs of different days was also observed when compared to the other groups.
Conclusion: Our results indicate that 5 % UCB -PL can be used as a supplement in MNC cultured cells and in cord blood hematopoietic stem cells for cell therapy purposes.
Key words: Proliferationand, differentiation, platelet Lysate derived from umbilical cord blood, Colony Assay

Peripheral Blood Stem Cell Apheresis in Small Children

Hedayati Asl A, Emam Jomeh M, Dinarooni P, Fallah V, Zangooei R, Maleki M

Mahak Cancer Children's Hospital, Tehran, Iran
Department of Pediatrics Stem Cell Transplantation

Objective: In low-weight children with cancer and healthy donor children, peripheral blood progenitor cells (PBPCs) have largely replaced bone marrow as source of autologous and allogeneic stem cells in part because of their relatively easy collection. However, there is a concern regarding medical, psychosocial and technical difficulties in small children.
Patients and Methods: We retrospectively analyzed peripheral blood stem cell apheresis in 24collections. Twenty patients were with cancer (11 patients=Neuroblastoma, 4 patients=Retinoblastoma, 3 patients=Germ cell tumor, 1 patient=Hepatoblstoma, 1patient=Wilm's tumor) and 4 healthy children donors. The study was conducted between 2012 and 2014. Peripheral stem cell apheresis was performed in the Mahak cancer children's hospital in a nice room for children where the patients stayed with their families. Patients were not routinely sedated. PBPC were collected by a COBE Spectra cell separator (COBE, Denver, CO, USA). Harvesting was performed after 5days mobilization.
Results: Mean body weight was 12.6 kg (range 8.9 kg–15 kg) for a median age of 3.3 years (range 1.1–5 years). Mean duration of harvesting was 210 min (range 174 –274 min).Mean volume of stem cell collection was 145 ml (rang 120 ml -250 ml). The mean number of total nucleated cells collected was 5.8 $x10^8$/ kg (range 3.3–8.9 x 10^8 /kg recipients). No side effects occurred. Children didn't require an additional haematopoietic progenitor mobilization or additional apheresis in other day. PBSC collection was without transfusion in healthy donor children.
Conclusion: PBSC collection may be difficult in small children owing to the large volume apheresis compared to the child's weight. Various problems, such as metabolic or haemodynamic disorders may be were seen. Peripheral Stem cell harvest can be performed in low-weight children under safe and effective conditions even when systematic priming by blood is avoided. Processing with increase of blood volume may to increase in the yield by recruiting progenitor cells.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Hematopoietic Stem Cell Transplantation for Childhood Leukemia in Mahak

Hedayati Asl A, Tashvigi M, Fallah V, Mehrvar A, Zangooei R, Olaad E, Naderi A, Dinarooni P, Taghavi N, Dalir E, Amirian S,

Mahak Cancer Children's Hospital, Tehran, Iran
Department of Pediatrics Stem Cell Transplantation

Objective: The cure rate of childhood acute lymphoblastic leukemia (ALL) has improved considerably and approaches 80% today. However, the outcomes of patients who suffer from leukemic relapse remain unsatisfactory. Despite the high cure rate of children and adolescents with ALL a subgroup of patients benefit from allogeneic HSCT. Allo HSCT remains the standard treatment for intermediate/high risk AML patients.

Patients and Methods: Twenty-nine patients, ALL= 20 and AML=9, age 1 to 20 years with median age 13 years, M/F = 16/13(M/F ALL=13/7, AML=3/6) underwent SCT in our hospital (from 2012-2014).Twenty-eight patients transplanted Allo HSCT and 1pt AML Auto HSCT. Conditioning regimens consisted of Busulfan (IV) + Cyclophosphamide for Allo and Cyclophosphamide + VP16 +Cytarabine for Auto HSCT. Peripheral blood (PB) was the source of progenitor cells in 24 patients, Bone marrow (BM) in 4 patients and cord blood in one patient. In Allo HSCT, 26 patient transplanted 6/6 matched and 2 pateints 5/6.GVHD prophylaxis regimen was cyclosporine + Mtx. All patients engrafted.

Results: In allogeneic PBSCT ALL patients' median time to absolute neutrophil count (ANC) > 0.5 × 10^9/L was 10 days, and the median time to platelet count > 20 × 10^9 was 12 days vs 18 and 20 days in Allo BM ALL patients. In allogeneic PBSCT AML patients median time to ANC> 0.5 × 10^9/L was 13 days, and the median time to platelet count > 20 × 10^9 was 15 days. (All patients with AML transplanted with PB). At present 25 pts are alive (18 ALL, 7 AML) and 4 pts died due to VOD, hemorrhagic stroke and relapse. TRM was 4.6% at 100 days. Median time of death after transplantation was 187 days in ALL and 195 in AML. In Allo PBSCT ALL patients hospitalization period were 33 days vs 42 in Allo BM ALL patients. Acute GVHD appeared in 85% pts. Chronic GVHD appeared in 45% pts. With a median follow-up of 15 months (3-30 months) after transplant the event-free survival were 79% and two years overall survival 85.9% in ALL patients. A median follow-up of 16.5months (6-26months) after transplant the event-free survival were 75% and two years overall survival 76% in AML patients.

Conclusion: Hematopoietic stem cell transplantation can lead to durable remissions in children and adolescents with leukemia and increase in survival of children. PBSCT in childhood ALL was consistent with significant faster ANC and platelet recovery in allogeneic PBSCT, hospitalization was shorter. Longer follow-up is required to evaluate fully efficacy and long-term results.

Autologous Stem Cell Transplantation for Children and Adolescents with Relapsed and Refractory Hodgkin's Lymphoma

Hedayati Asl A, Mehrvar A, Zangooei R, Faranoush M, Dinarooni P, Olaad E, Jenabzadeh M, Athari G, Fallah V, Haghighi M

Mahak Cancer Children's Hospital, Tehran, Iran
Department of Pediatrics Stem Cell Transplantation

Objective: Despite the generally excellent prognosis of children and adolescents with Hodgkin's lymphoma, approximately 20% of patients relapse. High-dose chemotherapy followed by autologous stem cell transplantation (ASCT) is a recognized treatment option for patients with relapsed Hodgkin's lymphoma. This study evaluates the results and outcome of non-cryopreserved autologous stem cell transplant of 23patients with Hodgkin lymphoma.
Patients and Methods: Twenty-three patients age 5 to 25 years (median 14.5 years, M/F = 17/6), with relapsed, refractory or poor prognosis HD, underwent ASCT in our hospital (from 2012-2014). Status at transplant was: second complete remission (CR2): *n* = 13; further CR (CR >2): *n* = 8, partial remission (PR): *n* = 2. Twenty-three patients received chemotherapy-based conditioning regimens: cyclophosphamide, carmustine and etoposide (CBV): 6, CCNU, etoposide, cytarabine and melphalan (CEAM): 17, Peripheral blood (PB) was the source of progenitor cells in 23 patients. All patients engrafted.
Results: The median mononuclear cell dose was 5.4×10^8/ kg. The median time to reach absolute neutrophil count > 0.5×10^9/L was 11 days, and the median time to platelet count > 20×10^9 was 13 days. Grade 2 and grade 3 mucositis was seen in 60% our patients. Transplant-related mortality at 100 days not occurred. Only two patient relapsed 15 and 18 months after transplant (mean 16.5 m.). With a median follow-up of 19.5 months (5-29 months) after transplant the event-free survival were 85%. All patients remain alive. No significant different between CBV group vs. CEAM group in engraftment day.
Conclusion: High-dose therapy with stem cell rescue can lead to durable remissions in children and adolescents with advanced HD. Future investigations should focus on strategies designed to decrease relapse after auto-transplantation, particularly in patients at high risk for relapse. Our analysis suggests that these regimens (CEAM, CBV) are feasible in pediatric patients with acceptable engraftment and toxicity.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Hypoxia Preconditioning As An Empowered Strategy For Improving umbilical Cord Blood Mesenchymal Stem Cell Viability

Asadpoor Dezaki Z, Kheirandish M*

Department of Immunology, Blood transfusion Research Center, High institute for Research and Education in Transfusion Medicine, Tehran, Iran.

The properties of Mesenchymal stem cells (MSCs) make them a potential candidate for cell therapy. In recent decade, human umbilical cord Blood derived mesenchymal stem cells (hUCB-MSCs) provide enormous potential for appropriate cell therapy in different fields. Researches have shown that hypoxia preconditioning (HPC) of bone marrow derived Mesenchymal stem cells (BM -MSCS) enhance their survival and extend their life span and inhibit pro inflammatory chemokines in MSCS. In this investigation, we examined the proliferation capacity of hUCB-MSCs under hypoxic condition in comparison with normoxic status and we introduced the best time of being under hypoxic conditions in contrast to the 24, 48 and 72 hours of hypoxia.
In This study hUCB-MSCs were isolated by Ficoll density. The Ethical approval was obtained from the institutional ethical review board at Blood Transfusion Research Center. The isolated populations have been characterized as mesenchymal stem cells by surface markers analysis and functional properties. UCB -MSCs were exposed to HPC protocol consisted on 2 sets of hypoxia (15 min, 2.5% oxygen (O2)) and reoxygenation (30 min, 21%O2), prior to hypoxic challenges (24, 48, 72 h). Cell survival was measured by the trypan blue staining. Occurrence of apoptosis was measured with MTT assay. Doubling time and growth rate were calculated in hypoxic and normoxic groups. In order to assess the role of hypoxia on surface markers expression, cells were analyzed by Flow Cytometry .The results showed that HPC status increased cell proliferation of UCB-MSCs in comparison with control group. The MTT results showed that cell viability of hUCB-MSCs after hypoxia preconditioning significantly increased in comparison with normoxic group .Our results suggested that hypoxia preconditioning can provide a suitable culture condition for rapid proliferation with no effect on their immunophenotype features. However, HPC - UCB-MSCs could be a potential therapeutic avenue for increasing the efficacy of stem cell therapy and support future application of UCB-MSCs for regeneration medicine.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The 1st International and 5th National
Congress on Stem cells
January 15-17, 2015; Tehran , Iran

Effect of Human Th17 Polarizing Factors on Morphologic Profile and Function of Mesenchymal Stem Cells

Arash Pourgholaminejad[1], Fattah Sotoodehnejadnematalahi[2], Nasser Aghdami[2], Seyed Mohammad Moazzeni[1]

[1]Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
[2]Department of Regenerative Biomedicine and Cell Therapy Group of Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

Introduction: Interleukin 17 (IL-17)–producing T helper 17 cells (Th17 cells) have been described as a T helper cell subset distinct from T helper type 1 (Th1) and Th2 cells, with specific functions in chronic inflammations, autoimmunity and transplant rejection. Interleukin-1β (IL-1β), IL-6 and IL-23 are the polarizing cytokines essential for human Th17 differentiation and leading inflammatory conditions. Mesenchymal stem cells (MSCs) have generated a great deal of excitement and promise as a potential source of cells for cell-based therapeutic strategies in inflammatory disorders and auto-immune diseases due to the immunomodulatory functions. Some studies have been reported that phenotypic profile and function of MSCs changed after exposure to pro-inflammatory conditions. Also, cytokines that induce differentiation of human Th17 lymphocytes, may cause conversion in MSCs profile and effector functions. Therefore, the aim of this study is to evaluate the effect of human Th17 polarizing factors (IL-1β, IL-6 and IL-23) on morphologic and function of MSCs.

Materials and Methods: Human Bone Marrow-derived MSCs (BM-MSCs) and Adipose-derived MSCs (AD-MSCs) were cultured in α-MEM containing 10% FBS, 1% L-glutamine and 1% NEAAs and treated with IL-1β (20 ng/ml), IL-6 (40 ng/ml) and IL-23 (20 ng/ml) for three days. Thus, MSCs were harvested and phenotypic markers (CD44, CD73, CD90, CD105, CD11b, CD34, CD45, HLA-DR, CD40, CD83, CD86) and multi-lineage differentiation capacity (Osteogenic and Adipogenic differentiation) were measured by Flowcytometry and Specific staining protocols, respectively. At last, the immuno-suppressive function of MSCs were analyzed by their effects on Mixed Lymphocyte Reaction (MLR) assay.

Results: In this study we showed pro-inflammatory cytokines IL-1β, IL-6 and IL-23 that polarize human Th17 cells showed no influence on expression of MSCs surface markers (CD44, CD73, CD90, CD105, CD11b, CD34) except CD45, both AD-MSCs and BM-MSCs were CD45+ after cytokine treatment. Also, we observed no effect on expression of co-stimulatory molecules (CD40, CD83, CD86) and HLA-DR. Adipogenic and osteogenic differentiation capacity were increased significantly in cytokine-treated MSCs. Moreover,

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

MSCs treated with cytokines were capable to suppress proliferation of stimulated lymphocytes even more than non treated cells.
Discussion: Based on these results, we concluded that human Th17 polarizing factors that promote inflammatory response in autoimmune and transplant rejection, could enhance multi-lineage differentiation and immunoregulatory capacity of mesenchymal stem cells. Finally, It's estimated that treatment of MSCs with pro-inflammatory cytokines will up-regulate their efficiency in cell therapy approaches.

The 1st International and 5th National Congress on Stem cells
January 15-17, 2015; Tehran , Iran

Study on Feasibility of Differentiation of Mesenchymal Stem Cell Derived from Human Dental Pulp into Pancreatic Islet-Like Cell

SamanehAltafi[1],Mohammad Mehdi Yaghoobi[2], HosseinAli Sasan[1]

[1]Department of Biology, Faculty of Sciences, ShahidBahonar University of Kerman, Iran
[2]Research Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran

Introduction: Recently, the generation of Insulin-Producing Cells (IPCs) from stem cells is highly regarded for the treatment of type 1 diabetes mellitus (T1DM). Human dental pulp stem cells (DPSCs) are considered an appealing source of mesenchymal stem cells (MSCs), since they are non-controversial, readily accessible, have a large donor pool, and pose no risk of discomfort for the donor. In this study, we explored the ability of DPSCs to differentiate into IPCs.
Materials and methods: DPSCs were extracted from human third molar and cultured in alpha MEM supplemented with 10% FBS. Then they were induced to differentiateinto pancreatic β-cells by a multistep procedure. The main components of the induction medium weretrans-retinoic acid, β-mercaptoethanol, nicotinamide, zincsulphate and selenium. Islet-like structures were assessed in DPSCs following21 days of treatment by differentiation culture. Expression of IPC specific markers Pancreatic and Duodenum Homeobox-1 (Pdx1), NK6 homeobox 1(Nkx6.1) and forkhead box O1genes (Foxo1)was analyzed at 5-7 days interval by real-time RT-qPCR.
Results: Human dental pulp stem cells form islet-like structures after 21 days of induction of differentiation. Upon multistep-IPCs induction, Real-timeRT-qPCR resultsconfirmed the expression ofPdx1, Nkx6.1 and Foxo1in differentiated cells.
Discussion: The present study demonstrates the potentialof humandental pulp stem cells to differentiate into insulin-producing cells.Therefore, upon further analysis in vivo, DPSCs may be considered for future cell therapy of pancreatic disorders.
Keywords: β-Cells, Diabetes, DPSCs, Insulin

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Matrilin-2 Upregulation in Cocultured Adipose Stem Cells with Chondrons

H. Shafaei PhD [1,3], M.N. Helder PhD [2,3], B. ZandiehDoulabi PhD [3] ,J. Klein Nulend PhD[3]

[1]Department of Anatomical Sciences, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Department of Orthopaedic Surgery, VU University Medical Center (Vumc), Research Institute MOVE, Amsterdam, The Netherlands.
[3]Department of Oral Cell Biology, Academic Center of Dentistry Amsterdam (ACTA), Research Institute MOVE, Amsterdam, The Netherlands.

Objective:Adipose stem cells (ASC) are used for cartilage tissue engineering.Among mesenchymal stem cells (MSC), ASC are attractive because of accessibility, abundance and rapid growth. Coculturing of ASCwith chodron(chondrocytes enveloped by their natural pericelluar matrix)may induce chondrogenic differentiation by expression of multiple cartilage specific molecules. Matrilin-2(MATN2)is animportant moleculein cartilage development but it is not evaluatedin in vitro chondrogenisof MSC yet. Therefore we investigatedchondrogenic potential of chondron to induce differentiation in ASC and also compared MATN2 gene expression with other cartilage biomarker.
MethodsandMaterials:Interactions between chondrons and ASC, both cultured in a micromass configuration to facilitate chondrogenic induction, were studied in a transwell system. ASC andchondronmicromasses were cultured as a control. The cocultures, ASC and chondroncontrols were cultured for 4 and 14 days. Gene expression of main chondrogenic and hypertrophic markers was analyzed by real-time RT PCR.
Results:chondrons significantly ($p<0.05$)unregulatedexpression of collagen type II and link protein but notaggrecangene expression in cocultured ASC in comparison to control ASC after 14 days. However in cocultured ASC, MATN2 more significantly ($p<0.0005$) expressed than ASC at day 14. Our results showed that expression of MATN2 in chondron from day 4 to day14 did not decreased instead of other genes.
Conclusion:Soluble factors secreted by chondronsinduceexpression of cartilage specific genes speciallyMATN2that indicateschondrogenic differentiation of ASC into chondrocyte has been occurred.
Keywords: Cartilage tissue engineering; Chondrogenesis; Chondron; Coculture: Matrilin-2

Investigation of *KLF4* Gene Expression as a Stemness Molecular Marker in Hair Follicle Stem Cells

Fatemeh Khaksar*[1], Saber Zahri[2], Asadollah Asadi[3].

[1]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: f.fatemeh77@gmail.com
[2]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: zahri@uma.ac.ir
[3]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: a_asadi@uma.ac.ir

Introduction: According to the source of stem cells, two types of the cells, including embryonic and adult stem cells have been introduced. Stem cells from hair follicle are adult stem cells. Considering that hair follicle stem cells are abundant and easily accessible at any age and not be rejected by the immune system, this type of stem cells are the best alternative source of stem cells. Pluripotency of embryonic stem cells (ESCs) were controlled by several transcription factors such as klf4.Expression of embryonic stemness markers in adult stem cells indicating remarkable proliferative potential of these cells. Klf4 is required for both ES cell self-renewal and maintenance of pluripotency and that the expression of Klf4 prevents ES cell differentiation in response to withdrawal of leukemia inhibitory factor (LIF) or bone morphogenetic protein 4 (BMP4). In addition, Klf4 directly binds to the promoter region of Nanog and regulates its expression. Materials and Methods: In this investigation detection of stem cell characteristic of hair follicle stem cells performed by amplification and detection of klf4 gene expression. In this study bulge cells of rat hair follicle were isolated and cultured according to previously published method. Then total RNA was extracted from various subcultures and the cDNA synthesis was performed using oligo(dT)18. The first strand cDNA was used as template for amplification of the genes. Results: The results of this study showed that hair follicle stem cells were klf4 positive.Discussion: Result showed that hair follicle stem cells have high proliferative potential and express the stem cells genes thus this type of cells are suitable alternative to therapist application and they can be introduce as potentialy active therapatic cells.
Keywords: adult stem cells, hair follicle, Klf4, Nanog

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The Study of Quantitative Expression Analysis of Pluripotency Genes, Nanog and Klf4, in Hair Follicle and Bone Marrow Derived Stem Cells

Fatemeh Khaksar*[1], Saber Zahri[2], Asadollah Asadi.[3]

[1]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: f.fatemeh77@gmail.com
[2]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: zahri@uma.ac.ir
[3]Department of Biology, Faculty of Science,Mohaghegh Ardabili University, Ardabil, Iran; email: a_asadi@uma.ac.ir

Introduction: Despite some progress advance in the field of stem cells cultivation, the choice of suitable and efficient source of stem cells were one of the main area of contention. Since,quantity of the pluripotency gene expression in the stem cells indicative their stemness potential, in this study expression of the pluripotency genes such as klf4 and nanog were studied as key factors in embryonic and adult stem cells, in stem cells drived from bone marrow and hair follicle.
Materials and Methods: in this study, the bulge cells of hair follicle and bone marrow mesenchymal stem cells were isolated and cultured. Total RNAswere reverse transcribed into cDNA using oligo-d(T)18 primers. The primers ofklf4, nanog and βactin (as a housekeeping gene), were designed by Oligo v7.o software and quantitative expression of the genes in two source of stem cells were recorded by Cyber green Real Time PCR.
Result: Two types of isolated stem cells efficiently express nanog and klf4 genes. However, nanog gene expression in the bone marrow mesenchymal stem cells significantlymore than hair follicle stem cells, while, the expression level of klf4 gene in hair follicle derived stem cells considerably were high.
Discussion: according to the expression of stemness genes in hair follicle derived stem cells, this type of cells can be introduce as multiple cells with high efficiency potential in restoration and differentiation.
Keywords: stem cells, hair follicle, Klf4, Nanog, Real Time PCR

Cardiac Repair Using by Human Umbilical Cord Matrix-derived Cardiomyocytes and Vascular Endothelial Growth Factor (VEGF)

Moradi Kaveh[1], Abbasi Mehdi[1]*, Aboulhasani Farid[1], Rastegar Tayebeh[1], Asgari Hamidreza[1], Abbasi Majid[1], Latifpour Mostafa[1], Koruji Morteza[2], SargolzaeiAval Fereidon[3], Salehi Majid[4] and Mohammadi Rad Mosleh[5]

[1]Department of Anatomical Science, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2]Cellular and Molecular Research Center & Department of Anatomical Science, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]Department of Anatomical Science, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[4]Medical Nanotechnology Department, Tehran University of Medical Sciences, Tehran, Iran
[5]Institute of Biochemistry and Biophysics, University of Tehran , Tehran, Iran

Objectives: In the previous study, although it has been shown that intramyocardial injection of human umbilical cord matrix stem cell (hUCM) improved cardiac function 4 weeks post MI, but angiogenesis has not been observed. Angiogenesis and replacing lost cardiomyocytes with new, live cardiomyocytes are considered as two key agents in cardiac repair. To achieve the above two factors we examined the effects of combination of stem cell and angiogenic therapy approaches by simultaneously injection of hUCM-derived cardiomyocytes with vascular endothelial growth factor
(VEGF) in cardiac repair.
Methods: MI-induced animals(by ligation of LAD) received 50 µl PBS, 5 × 10^6 differentiated hUCM cells (dhUCM), 5µg VEGF in normal saline and 5 ×10^6dhUCM cells combined with 5µg VEGF in normal saline, intramyocardialy. MI group, with no other intervention, served as a control group. We were assessed survival, migration and integration of dhUCM cells, as well as angiogenesis eight weeks post MI induction.
Results: Eight weeks post MI, although dhUCM and VEGF groups have shown that LVEF and LVFS improved significantly, but animals in dhUCM+VEGF group have the highest rise in LVEF and LVFS in comparison to the other MI-induced groups ($p<0.05$). Histological and morphological analysis have revealed that myocardium of animals in dhUCM+VEGF group have the highest vascular density and the lowest fibrosis tissue in comparison to the other MIinduced groups ($p<0.05$). Immunohistological assessments revealed that transplanted dhUCM cells have survived, migrated to infarcted area and integrated with recipient cardiac tissue.

Conclusion: we have found that intramyocardial administration of dhUCM cells combined with VEGF improved cardiac function, enhanced angiogenesis and reduced fibrosis tissue formation after MI, eight weeks post MI.

Keywords: Human Umbilical Cord Matrix-derivedcardiomyocytes, Myocardial infarction, Angiogenesis, Vascular endothelial growth factor, Cell therapy, Cardiac repair, Rabbit.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The 1st International and 5th National
Congress on Stem cells
January 15-17, 2015; Tehran , Iran

Development of Monoclonal Antibody Against Hematopoietic Stem Cell Antigen CD34

Leili AghebatiMaleki *,Ali AghebatiMaleki

Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction: CD34 is highly glycosylated surface antigen of enormous clinical utility in the identification, enumeration, and purification of engraftable lymphohematopoietic progenitors for transplantation. The treatment of hematologic malignancies, and immunodeficiency is offered by hematopoietic stem cells (HSCs) as a unique self-renewal and differentiation source which most commonly is selected by CD34 surface marker for HSC. The prim aim of this study was to generate mAb against CD34 useful in purification of human hematopoietic stem cells.
Materials and methods:Balb/c mice were immunized with CD34 and Spleen cells were fused with SP2/0. Fused cells were grown in hypoxanthine, aminopterine and thymidine (HAT) selective medium and cloned by limiting dilution. Large scale of monoclonal antibodies was produced by mouse ascites production of mAb (in vivo) method. Monoclonal antibody was purified by chromatography. Then reactivity of these antibodies was evaluated in different immunological assays including ELISA, immunofluorescence (IF), western blot (WB) and flow cytometry.
Results: In this study, between five positive clone wells, two clones were chosen for limiting dilution. Limiting dilution product was one monoclone (3-D5 monoclone) with absorbance about 2. Isotype of this mAb was identified as IgG1 class with Kappa (κ) light chain.
Discussion: Anti-CD34 monoclonal antibody could apply in diagnosis of hematologic malignancies, solid tumors, and immunodeficiency diseases that offered by hematopoietic stem cells (HSCs), isolation of hematopoietic progenitor cells, disease monitoring, and in vitro differentiation studies. Also, anti-CD34 mAb may represent a powerful tool for the positive selection or depletion of cells expressing human CD34 antigen. Upon our findings, it can be proposed that this particular approach for production of an anti-CD34 peptides antibody is feasible and cost-effective. Further, this study clearly indicates that the produced antibodies can be used in research, diagnosis as well as clinic if produced in chimeric form.
Keywords: Hematopoietic stem cell, Monoclonal antibody, CD34

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Generation and Characterization of anti-CD133 Monoclonal Antibodies Reactive with Hematopoietic Stem Cells

Leili Aghebati Maleki *

Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction:CD133, formerly known as AC133, is a marker that is frequently found on multipotent progenitor cells, including immature hematopoietic stem and progenitor cells. In the hematopoietic system, CD133 is expressed on a small portion of CD34– cells as well as on a subset of CD34bright stem and progenitor cells in human fetal liver, bone marrow, cord blood, and peripheral blood. CD133 has also been found to be expressed on circulating endothelial progenitor cells, fetal neural stem cells, other tissue-specific stem cells, such as renal, prostate, and corneal stem cells, cancer stem cells from tumor tissues, as well as ES and iPS cell-derived cells .The prim aim of this study was to generate mAb against CD133 useful in purification of human hematopoietic stem cells.
Materials and methods:Two peptides from extracellular domain of human CD133 were designed and selected as immunogen. The designed peptides were conjugated to KLH and BSA, separately. The peptide - KLH was used for immunization of four Balb/c mice (6-8 weeks old). The most immunized mouse was selected for fusion by ELISA technique. Supernatants of hybridoma cells were screened by ELISA method and suitable clones were selected for limiting dilution. Specificity and cross-reactivity of the mAbs were determined by sandwich ELISA, and Western blot analysis.
Results:In this study, the serum of the immune mouse at 1/8000 dilution, displayed the high absorbance in reaction with BSA-peptide using indirect ELISA. The immune mouse was selected for the fusion and their spleen cells fused with myeloma SP2/0 as fusion partner. The fused cells were suspended in HAT medium and distributed into five culture plates containing feeder layer.Several anti-CD133 monoclonal antibody producing hybridomas were obtained.
Among them one clone had a high reactivity with immunogenic peptide in ELISA assay. The purity of purified antibody was assessed by SDS-PAGE. A single band of about 150 kDa in SDS -PAGE analysis indicated the proper purification of the antibody. Western blotting technique was performed to see the pattern of reactivity of anti- CD133 monoclonal antibodies with different cell lines such as HUVECs, KG1a and WERI-Rb-1 cells that only one specific band was seen in 120 kDa in WERI-Rb-1 lysate and also was used for confirming the result of ELISA.
Discussion: The identification and isolation of human hematopoietic cells expressing CD133, combined with use of in vitro and in vivo assays, has provided novel insights into the hematopoietic progenitor and stem cell compartment in the human. AC133 antibody

provides an alternative to CD34 for the selection and characterization of cells necessary for both short- and long-term engraftment, in transplant situations, for studies of ex vivo expansion strategies, and for gene therapy.CD133 antibodies are useful reagents for the isolation of hematopoietic and endothelial progenitor cells.

Keywords: Hematopoietic stem cell, Monoclonal antibody, CD133

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Induction of Differentiation by miR-124 and miR-128 in Glioblastoma Cancer Stem Cells

Samaneh Sharif[1], Mohammad Hossein Ghahremani[1,2], Masoud Soleimani[3]

[1]Department of Molecular Medicine, School of Advanced Technologies in Medicine,Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Toxicology & Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
[3]Department of Hematology, Tarbiat Modares University, Tehran, Iran.

Glioblastoma is considered as one of the most aggressive brain tumor. Detailed studies show that this kind of tumor is resistant to conventional therapies since there is often a subset of cells that has the potential of initiating and maintaining the growth of gliomas which are known as the glioblastoma cancer stem cells (CSCs). CSCs have the capacity of renewing themselves and producing the heterogeneous lineage of cancer cells. In recent years, CSC has become one of the main therapeutical targets in management of glioblablastoma. One of the newest therapeutic ways for controlling the tumors is differentiation of CSCs. A set of endogenous non-coding RNAs that play a crucial role in regulating development processes are microRNA (miRNAs). miR-124 and miR-128 are the most specific neural lineage miRNAs in brain of adults and patients suffering glioblastoma have downregulation of these miRNAs.
The purpose of this study was to induce differentiation of CSCs by overexpression of miR-124 and miR-128 in CSCs. To this end, U87 glioblastoma cell line was incubated in serum-free neural stem cell medium and the forming neurospheres were collected using the magnetic-activated cell sorting (MACS) method to isolateCD133 positive cells. These cells were transfected with miR-124 and miR-128 and the expression of neural and neural stem cells (NSC) markers were detected by qPCR and immunostaining. Our studies showed that the treatment of CSCs with neural differentiation and maturation miRNAs resulted in significant increase in β-tubulin III and GFAP (neural markers expression) while reducing nestin (NSC markers). Furthermore, miR-124 and miR-128 inhibited the proliferation of glioblastoma tumor cells and decreased their migration. Therefore, these microRNAs can differentiate the CSCs and reduce the recurrence of tumors after treatment.

Transplantation of Human Embryonic StemCell Derived Midbrain Dopaminergic Progenitors into the Rat Model of Parkinson's Disease

Dolatyar B[1,2], Fathi A, Salekdeh GH, Javan M, Baharvand H.

[1]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[2]Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran.

Human embryonic stem cells (hESCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy in Parkinson's disease.In the present study we used a defined method to generate human DA progenitor cells from human embryonic stem cells that is based on dual SMAD inhibition and embryoid body (EB) formation. Our results showed that the human DA progenitor cells expressed the midbrain DA progenitor markers such as FOXA2, CORIN, LMX1A and LMX1B, and can be successfully differentiate into TH^+ mature neurons. We surveyed the proteome of the human DA progenitor cells for novel surface proteins in previous study which resulted in expression of CNTN2, FLOT2 and TAG1. Flowcytometry analysis of these cells indicated that they express 20% CNTN2, 17% Flot2, 11% Tag1 and 22% CORIN. To evaluate the functionality of these cells we transplanted $CNTN2^+$ cells on day 12 post neural induction into 6-OHDA-lesioned rats and also transplanted unsorted human DA progenitor cells and Human Dermal Fibroblasts (HDFs). The behavioral results of motor performance tests such as apomorphine-induced rotation and cylinder test showed that unsorted human DA progenitor cells can significantly improve motor behavior2 weeks after transplantation, although HDF cells didn't make significant behavioral improvement until 12 weeks after transplantation. Behavioral data from animals transplanted with $CNTN2^+$ cells are under investigation. Our method is favorable in terms of efficiency and safety and indicate promise for the development of cell-based therapies in Parkinson's disease.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Over Expression of HIF-1α Gene Increases the Production of Stem Cell Factor in Human Mesenchymal Stem Cells and Increases their Supportive Functions for Hematopoietic Stem Cells

Ali Asghar Kiani[1], Mohammad Soleyman Soltanpour[2], Ehsan Aali[3], Ali Shakerizadeh[4], Masoumeh Kashi[5]

[1]Department of Hematology and Blood banking, School of Medicine, Lorestan; University of Medical Sciences, Khorramabad, Iran. aliasgharkianii@gmail.com/ 09166638354
[2]Department of Hematology and Blood banking, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
[3]Department of Pharmacology, School of Medicine, Qazvin University of Med. Sciences, Qazvin, Iran.
[4]Department of Medical physic, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
[5]Faculty of Health, LorestanUniversity of Medical Sciences, Khorramabad, Iran.

Introduction: Bone marrow transplantation is a critical approach to treatment of many hematological disorders. Co transplantation of efficient mesenchymalstem cells can greatly improve the transplantations. Hypoxia inducible factor-1alpha(HIF-1α) is one of the most important genes in the body and we taught this gene might change the efficiency of MSCs by affecting of the production of some cytokines. To address this issue, human MSCs were manipulated to over express HIF-1αgene, in this study.
Materials and Methods: Full-length cDNA of humanHIF-1α was entered into human bone marrow Mesenchymal stem cells by pcDNA.3.1non-viralplasmid vector (pcDNA-HIF-1α), and the effect of this over expression on production of some hematopoietic growth factors in both normoxia and hypoxia was studied.In co-culture ofHIF-1α over expressed MSCs(MSCS-HIF-1α) with hematopoietic stem cells, the effect of this over expression on HSCs also was evaluated.
Results: Over expression of HIF-1α in MSCs in normoxia caused increased production of one of the most important hematopoietic growth factors ,Stem cell factor. The increased expression had no effect on production of other hematopoietic growth factors that we evaluated in this study. In co-culture of MSCs-HIF-1α with HSCs, increased colony formation and reduced differentiation of HSCswas observed.
Conclusion: Increased expression of HIF-1α in human MSCs can augment the production of some hematopoietic growth factors, which can herald more effective role of MSCs in cell therapy specially bone marrow transplantation.
Keywords: HIF-1α, MSC, SCF, pcDNA

Expansion of Human Umbilical Cord Blood Hematopoietic Stem Cells Using Small Molecules

Morteza Zarrabi, M.D,[1,2]Elaheh Afzal, MSc,[1] Marzieh Ebrahimi*,PhD,[2]

[1]Royan Stem Cell Technology, Cord Blood Bank, Tehran, Iran
[2]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

Introduction: Despite the great potential of cord blood hematopoietic stem cells (CBHSCs)for clinical applications, obstacles such as their relative immaturity and limited number of them in a single cord blood unit- which in turn result in graft failure and delayed recovery- have limited the use of cord blood HSCs in cell therapy. Ex vivoexpansion of HSCs is a solution to overcoming these limitations.So,after investigation the most important signaling pathways in HSCs self-renewal, we haveinitiated a study using small molecules compounds. These naturally or artificially organic compounds can help us in regulating biological processes in a controlled and specific manner and investigation the new target molecules and signaling pathways. Our selected small molecules are: SB431542, CHIR99021, bpv, Nicotinamide (NAM), Purmorpamine (Pur), Pifithrin μ (Pμ).
This combination of small molecules could not only support the proliferation (SB, Pur) and survival pathways (bpv), but also inhibit the differentiation (CHIR99021) and apoptosis (Pμ) pathways.
Materials and methods: Isolation of CB mononuclear cells:For isolation of mononuclear cells (MNCs), the leukocyte-rich fraction of cord blood was layered on Ficoll.
Ex vivo cultures:Human CB MNCs (106/ml) were cultured in in various conditions:culture condition A, supplemented neither bycytokines (SCF, FL, TPO) nor by small molecules. Culture condition B, supplemented only with cytokines.Culture condition C, supplemented with cytokines and small molecules.
Cell Characterization: In order toevaluate the HSCs expansion,the total nucleated cell count (TNC), colony-forming cell assay, analysis of CD34+CD38- expression by flow cytometry, and gene expression of amplified cells were determinedon Day 10 after culture.
Cell expansion was expressed as fold increase, calculated by dividing the output number of cells,CD34+CD38- cells and colony forming cellsafter amplification cultures on day 14 by the respective input cell number on day 0.
Results: Based on our initial results the fold increases of TNC,CD34+CD38- cells and colonyforming cells in different groups are as follows: TNC (A: 0.2, B: 1.5, C: 4.7), CD34+CD38- cells (A:0.1,B:1,C:1.2), colony forming cells (A:0.17, B:1.56, C:1.86). Furthermore, the Realtime PCR analysis relative to negative controlshows significant increases in the expression of Stemness marker genes like HOXB4, BMI1 and GATA2 in culture condition C.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Conclusion: Although further investigations are required, according to our initial results,the combination of mentioned small molecules could potentially increasethe expansion rate of cord blood MNCs ex vivo.

A Simple and Practical Method for Increasing of Proliferation the Mesenchymal Stem Cells in Mouse

Ali aghebatimaleki, leili aghebati Maleki

Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction: Lactobacillus extracts and supernatants have been used as probiotics in human and veterinary medicine for their ability to enhance wound healing and immunity. Previous datademonstrated that Lactobacillus supernatant (LS) stimulated wound healing, angiogenesis and proliferation of embryonic cells after topical application.Mesenchymal stem cells have pretty less growth and confined number of passages. In this study the supernatant survey of Lactobacillus acidophilus bacteria in terms of one of the most effective probiotics on the increasing proliferation of the mesenchymal stem cells due to enhance of the increasing them in the case of the treatment of the patients who need the graft by mesenchymal stem cells has been done.

Materials and methods :The survey has been done on mesenchymal stem cells separated from bone marrow of mouse that it leads the second passage to the divisible stage due to admission of their mesenchymal. Afterwards, the cells treated with Lactobacillus supernatant (LS) which has been separated beforehand in the second passage and the number of the cells was measured by MTT test equated with the standard. In addition, we also brought treatment ambiance of medium of MRS broth with cells disparately and the statistical results were surveyed.

Results: The curve of the cell's growth in different amounts was drawn and we converted the results of MTT test to the number of cell by standard curve. Then by using statistical analysis we could determine the rate of data with SPSS and their significancy with ANOVA. Our discovery represents the over effect of Lactobacillus supernatant (LS)on the light effect of the ambiance of medium of MRS broth.

Discussion:This study demonstrates that use of Lactobacillus supernatant (LS) is a practical and economical method for increasing and stimulating of proliferation themesenchymal stem cells which are isolated from bone marrow.

Keywords: Lactobacillus acidophilus, Mesenchymal stem cells, Proliferation

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Direct Conversion of Adult Human Dermal Fibroblast into Hematopoietic Cells

Fatemeh Ganji [1], Marzieh Ebrahimi [1, 2*], Hossein Baharvand[1]

[1]Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute forStem Cell Biology and Technology, ACECR, Tehran, Iran.
[2]Department of Regenerative Biomedicine at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran.

Direct conversion of fibroblast to another without an intermediate pluripotent stage is also possible but ,at present, requires the viral transfection of appropriate transcription factors which limits its therapeutic potential in future.In previous study was shown that Oct4 alone is sufficient to reprogram cells and it was found that Oct4 expression is required for differentiation of ESC into all three lineages. Moreover, generation of transgene free reprogrammed cells viaprotein transduction in combination with small molecules was investigated more by scientists due to safety issues. Our primary data have shown that brief treatment of human dermal fibroblast (HDF) with 5-aza (as a DNA methyltransferas inhibitor) can induce hematopoietic differentiation in combination with cytokines.We showed that combination of 5-aza and protein transduction (as safe approach) can induce reprogramming of HDF into hematopoietic cells effectively.In this case, HDFs were exposedfor 18 hours to 1µg 5-aza and added 200nM mL^{-1} Oct4 for 10 days. Cells were allowed to recover in embryonic stem cell medium for 3 h, and then followedby differentiation protocol that lasted 45 days.Our findings revealed that hematopoietic progenitors (CD34 and CD45) and erythroid genes (Hemoglobin α, β and γ) were expressed on 10 and 45 of differentiation, subsequently.Moreover,most of cells became roundon day 24 and about 50% of cells expressed hemoglobin clearly. These findings demonstrate that Oct4 in combination with 5-aza is sufficient to reprogram HDF to hematopoietic cells.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

The 1st International and 5th National
Congress on Stem cells

January 15-17, 2015; Tehran , Iran

Optimizing the Enzyme Activity for Isolating MSC-like Cells from Human Umbilical Cord Vein

Homa Salami[1], Ashraf Aleyasin[2], Seyed Javad Mowla[1]

[1]Department of Genetics, Faculty of Basic Sciences, TarbiatModares University, Tehran, Iran
[2]Department of Gynecology and Infertility, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Mesenchymal stem cells (MSCs) are multipotent adult stem cells. Extensive self-renewal and multilineage differentiation potential of MSCs offers them as a great promising source for cell-based therapies. First identified in bone marrow stroma, MSCs are also found in more available tissues such as lipo, umbilical cord, amniotic fluid and dental pulp.Due to the low frequency at which these cells occur in tissues it is desirable to optimize the isolation methods. Here we show that the activity of enzyme which is applied for isolation of MSCs from umbilical cord vein can be improved by modifying the solvent.
Matherials and methods: Human umbilical cords were collected and processed within 6-12 hours after normal deliveries with informed consent frommothers.For isolation we followed the protocol that is set forth by Romanovet al. collagenase IV 0.1% solution is made with 3 different solvents; PBS, Medium 199 and Medium 199 diluted with PBS. The umbilical cords are filled with one of the solutions and incubatedat 37ºC for 25 min. collected cells are cultured in 6-well tissue culture plate containing DMEM-LG,10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. The cellswere maintained at 37°C in humidified atmosphere containing 5% CO_2 and the culture medium is changed every 2 days.
Results: Fibroblastoid cells were observed in all 6 wells of the plate after 3 days. The number of MSC-like cells isolated from the first solution is higher than the two and the second solution has the lowest number of cells. The number of isolated cells in each well was sufficient for further proliferation.Although all cells could proliferate well, cells from the third digestion showed the highest proliferation rate in the first 10 days.
Discussion: Ca^{2+} plays a role of co-factor for activation of collagenase IV. Therefore Medium 199 which has a sufficient concentration ofCa^{2+} in comparison with DMEM is chosen as a solvent for the enzyme. On balance, cell culture mediums decrease the activity of the enzymesbutprovide better environment for newly detached cells.As the result of this experiment suggests, solving collagenase IV in the mixture of Medium 199 and PBS decrease the enzyme activity less than the second solvent as well as keeping the dissociated cells in fine environment.

Website: ishsct.org
Email: ishsct@ishsct.org
Tel: +982188220061-63

Assessment of the Effects of low-glucose Cultures on Freshly Isolated Human Umbilical Cord Vein MSC-like Cells

Homa Salami[1], Ashraf Aleyasin[2], Seyed Javad Mowla[1]

[1]Department of Genetics, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, Iran
[2]Department of Gynecology and Infertility, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Mesenchymal stem cells (MSCs) are of great potential in cell-based therapies with applications relating to tissue engineering, regenerative medicine, and gene therapy. MSCs occur at low frequency in tissues therefore it is essential to optimize expansion methods to obtain sufficient numbers for research and therapeutic applications. Here we investigate the effects of rich, low-glucose cultures on proliferation of freshly isolated human umbilical cord vein MSC-like cells.
Materials and methods: Human umbilical cords were collected after normal delivery with informed consent from mothers. The isolation of MSCs followed the method set forth by Romanov et al. Then the isolated cells were cultured in 24-well tissue culture plate with three different mediums; DMEM-LG, Medium 199 and DMEM-F12. For each Medium three different concentration of FBS, 10%, 15% or 20%, is added. All cultures contain 100U/ml penicillin, 100µg/ml streptomycin. The cells were maintained at 37°C in humidified atmosphere containing 5% CO_2 and fed with fresh medium every 2 days.
Results: MSC-like cells cultured in Medium 199 with 10% FBS showed well expansion and proliferation but cells other than fibroblastoid cells were observed to be able to expand in the culture. In comparison, less non-fibroblastoid cells were observed in wells containing DMEM-F12 with 10% FBS and after 2 weeks MSCs-like cells dominate the cell culture.
Discussion: Glucose is a primary fuel for animal cells. Freshly isolated cells usually fail to maintain and expand in low-glucose cultures for long period of time. MSCs, however, have the ability to proliferate in both low and high-glucose cultures. This ability is a valuable tool for having homogeneous cells in primary cultures. However, our result shows that the amount of glucose is not a key selector of fibroblastoid cells in primary culture of human umbilical cord vein. Although the moderate amount of glucose in DMEM-F12 in companion with 10% serum can result in having homogeneous MSCs-like cells culture which are newly isolated from human umbilical cord vein.