IRADIATION OF CELLULAR BLOOD COMPONENTS WITH COBALT 60 IS VERY EFFICIENT AND SAFE IN THE PREVENTION OF TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE (TA-GVHD) IN THE ALLOGENIC STEM CELL TRANSPLANT SETTING

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For the prevention of TA-GVHD in patients who received an allogenic stem cell transplant it is necessary to perform the gamma irradiation of all the cellular blood components. This irradiation is usually done with cesium 137 and with a special blood bank irradiators. However these devices are expensive; because that, in developing countries, is frequent the utilization of cobalt 60 and the same device that is used in the radiotherapy department, instead of blood bank irradiators. However the information about the efficiency and safety of this procedure is scarce. We present our experience with this technique.

From Dec 2002 to Dec 2005 thirty patients received an allogenic stem cell transplant and 28 were analysed. The stem cells source was: peripheral blood 23, unrelated cord blood 2, bone marrow 1.

The irradiation of the blood components was performed with cobalt 60. 1.24 MeV (theronation 780±3); the irradiation field was calculated for covering all of the bag surface and a dose of 3.5 Gy was administered to the mild plane of the bag. 158 blood concentrates were transfused, 68 red cell (X:2.5 per patient), and 90 platelets (3.2 per patient). The pre transfusion median hemoglobin and platelet levels were 7.63 g/dl and 12.000/ul; after transfusion was a median increase of 2.3 g/dl (0.6-4.7) in hemoglobin and 18.000/ul (0-140.000) in platelets.

There was no any case of TA-GVHD. Four patients developed pos transfusion aGVHD, in all of the cases the disease began 50 days or more after the last transfusion, there were no pancytopenia and the aGVHD was resolved completely with the treatment.

Conclusion:
In recipients of allogenic stem cell transplant the gamma irradiation of blood components with cobalt 60 and the same device which is used for patients radiotherapy is 100% effective and safe in the prevention of TA-GVHD. This is a very good alternative in centers without a blood bank irradiator.

SUCCESSFUL PHASE II TRIAL USING MESENCHYMAL STEM CELLS (MSC) IN COMBINATION WITH STEROID THERAPY FOR THE PRIMARY TREATMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE (AGVHD)

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AGVHD is a major cause of morbidity and mortality after allogeneic stem cell transplantation (SCT). Primary treatment of aGVHD with steroids achieves complete response (CR) rates of only 20-40%. MSC may provide effective GVHD therapy. In this study, Prochymal, an ex-vivo cultured MSC derived from unrelated donors, was used in combination with conventional steroid therapy for primary treatment of aGVHD. Pts were eligible if they had newly diagnosed aGVHD, grades II-IV, after undergoing related or unrelated SCT, or donor lymphocyte infusion (DLI). Study endpoints were drug safety and aGVHD response rates by day 28 after infusion. Pts were randomized to 2 doses of Prochymal: 2 (low) or 8 million (high) cells/kg. Prochymal was initiated along with steroid therapy at time of aGVHD diagnosis. 2 Prochymal infusions were administered 3-5 days apart within 72 hrs of steroid initiation. Tacrolimus, cyclosporine, or MMF were maintained for GVHD prophylaxis.

Pts were maintained on steroids (2 mg/kg/d) for at least 1 week with the objective of subsequently tapering off steroids. 32 pts (23 males, 9 females) were enrolled, and 31 were evaluable with median age 52 yrs (range 34-67). AGVHD developed following matched sibling (n = 15) or matched unrelated SCT (n = 13), or DLI (n = 4). Distribution of aGVHD is described in table below. Pts were randomized to low (n = 17) or high (n = 15) Prochymal dose. 90% of pts (n = 28) initially responded to aGVHD treatment. 21 achieved CR with no evidence of GVHD, and 7 achieved PR with a reduction in 1 organ stage. 100% of pts initially diagnosed with skin GVHD had a response to treatment, and 83% of pts with GVHD involving GI alone or with other organs had a response. 9 pts (31%) eventually required a second line agent to control aGVHD. Non-relapse survival at day 100 was 79.3% with 8 pts dying at a median of 48 days (range 13-58); aGVHD (n = 2), intracranial bleed (n = 1), relapse (n = 1), infection (n = 4). No inflammatory toxicities or discontinuation of treatment was observed. 1 pt developed atrial fibrillation 24 hrs following the second Prochymal infusion. No ectopic tissue formation was noted by CT scans at day 28. Addition of Prochymal to standard steroid therapy for the primary treatment of aGVHD resulted in a high response rate with minimal added toxicity. This trial demonstrates the potential of using a universal, cellular product for the treatment of aGVHD. A phase III clinical trial has been initiated to confirm these promising results.

Grade and Distribution of GVHD

<table>
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<th>Grade (no. pts)</th>
<th>LI(21)</th>
<th>II(8)</th>
<th>III(5)</th>
<th>IV(3)</th>
</tr>
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<tbody>
<tr>
<td>Organ (no. pts)</td>
<td>G1(12)</td>
<td>S0n(13)</td>
<td>G1/S0n(5)</td>
<td>G1/L0ver(2)</td>
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INITIAL SELECTION OF HIGH AFFINITY CD25+ CELLS INCREASES THE PURITY OF CD4+CD25+FOXP3+ T REGULATORY CELLS EXPANDED IN MEDIUM CONTAINING RAPAMYCIN

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CD4+ T regulatory cells (Tregs) with potent suppressor activity are marked by high levels of CD25 and expression of the transcription factor, FoXP3. We described an approach to isolate and expand Tregs using a single CD25-enrichment on a MACS column, activation by CD3/CD28-coated beads, and culture in medium containing rapamycin (Keever-Taylor et al, submitted). We achieved 10-fold expansion at day 10 of cells with potent suppressor activity that were enriched for CD4+CD25+FOXP3+ cells and 2) increase the dose of rapamycin to minimal added toxicity. This trial demonstrates the potential of using a universal, cellular product for the treatment of aGVHD.
TREATMENT OF STEROID REFRACTORY, SEVERE ACUTE GRAFT VERSUS HOST DISEASE WITH EXPANDED MESENCHYMAL STEM CELLS IN CHILDREN HAVING UNDERGONE ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Despite advances in pre-transplant immune suppression and donor HLA typing methods acute graft versus host disease (aGvHD) remains a significant problem following allogeneic HSCT. Steroid therapy is the treatment of choice in severe aGvHD. Steroid non-responsive aGvHD is associated with increased morbidity and more importantly death due to organ damage and/or infection related to the use of continuing immune suppression. Second line treatments continue to be evaluated. Whatever their initial effects, presently they have had little impact on overall survival.

Mesenchymal stem cells (MSCs) are poor antigen presenting cells, not expressing MHC class II or co-stimulatory molecules. They down regulate allo-reactive T cell responses when added to mixed lymphocyte cultures. MSCs alter cytokine excretion profiles of dendritic cells, naive and effector T cells, and NK cells inducing a more tolerant phenotype.

MSCs have been used successfully in a child with resistant aGvHD (Le Blanc, Lancet 2004).

We conducted an ethically committee approved prospective phase II/II study of co-infusion of expanded MSCs for treatment of children with steroid refractory, grade II-IV aGvHD. MSCs, isolated from parental donor marrow were expanded under GMP conditions. MSCs either as haploidentical or 3rd party.

Patient characteristics receiving MSCs for steroid refractory GvHD.

UPN 1 | UPN 2 | UPN 3 | UPN 4
---|---|---|---
Male | Male | Female | Male
MDS RC | Omens | Fanconi | JMMML
1y, 3mo | 1y, 4mo | 6yr, 10mo | 1yr, 7mo
Sibling ID | ORD ID x 2 | Matched UD | Matched Cord
GvHD 4 (Skin/Gl/Liver) | GI | chronic/plus | Gl plus Adv
CMV graft failure | Steroid/Tacrolimus/MMF | Steroid/CYT/ant CD25 | Steroid/Tacrolimus/MMF
MSC1 3rd party | MSC2 haplo | MSC1 3rd party | MSC2 3rd party
1.1 × 10^6/kg | 1.8 × 10^6/kg | 2.3 × 10^6/kg | 1.76 × 10^6/kg
Male 33 years | Female 25 years | Male 33 years | Female 25 years
2 infusions | 2 infusions | 2 infusions | 2 infusions
4–0 CR | No response | 4–0 CR | 4–0 CR
Died | Died | Alive Limited | Alive small
Klebsiella sepsis | CMV/GvHD | cGvHD skin | bowel fibrosis

UPN - Unique patient number; CR - complete response; PR - partial response; AdV - adenovirus; CMV - cytomegalovirus; EBV - Epstein Barr virus; HLH - hemophagocytic lymphohistiocytosis; MDS RC - myelodysplastic syndrome and refractory cytopenia; JMML - Juvenile myelomonocytic leukemia; CSA - cyclosporine

BLOTTING LFA-1 ACTIVATION WITH LOVASTATIN PREVENTS GRAFT-VERSUS-HOST DISEASE IN MOUSE BONE MARROW TRANSPLANTATION

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Leukocyte function associated antigen-1 (LFA-1) regulates T cell adhesion and activation. LFA-1 is constitutively expressed on cell surface in an inactive state. The control of LFA-1 activation is critical in inflammatory and immune responses. We demonstrated previously that the I-domain, the ligand binding site of LFA-1, changes from the low-affinity state to high-affinity state upon activation. Therapeutic antagonist, such as lovastatin, stabilizes the I-domain in the low-affinity state and inhibits the LFA-1 activation. Here, we report that lovastatin can block mouse T cell adhesion and proliferation in vitro. First, we demonstrated that lovastatin treatment reduced the mortality and morbidity in the mouse GVHD model. Lovastatin treatment significantly decreased GVHD mortality with 80% mice survived over 28 days, whereas more than 70% of the control mice died within the first 10 days, and the p values was 0.045. There were significantly reduced tissue damages in the skin, intestine and liver of lovastatin-treated mice. Second, we found lovastatin treatment reduced donor T cell homing to lymph nodes. There was a 65% reduction of CD4+ T cells homing to lymph nodes in lovastatin treatment group compared to control. The reduction of CD8+ T cells was greater with about 76% less cells homing to lymph nodes in the lovastatin treatment group. Third, we found lovastatin treatment reduced donor-derived T cell proliferation in vivo. There were 37% CD4+ and 31% CD8+ T cells remained undivided in the lymph nodes of the control mice at day 4 post-transplant. The lovastatin-treated mice had reduced proliferation kinetics of both CD4+ and CD8+ T cells with about 55% and 42% remained undivided. In the control lymph nodes, there were 42% CD4+ and 59% CD8+ T cells.