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IRRADIATION 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 ALLOGENEIC TRANSPLANT SETTING

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For the prevention of TA-GHVH in patients who received a allogeneic stem cell transplant is mandatory the gamma irradiation of the all 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 a allogeneic stem cell transplant and 28 were analysed . The stem cells source was: peripheral blood 25, unrelated cord blood 2, bone marrow 1. The irradiation of the blood components was performed with Cobalt 60 1.24 Mev- (theratron 780 C); 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 gm/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 transplant 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 receptors of allogeneic stem cell transplant the gamma irradiation of blood components with Cobalt 60 and the same device wich 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.

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

Grade (no. pts)	**II(21)**	**III (8)**	**IV(3)**	
Organ (no. pts)	**GI(12)**	**Skin(13)**	**GI/Skin(5)**	**GI/Liver(2)**

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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+Foxp3+CD27+ cells using 1-10 ng/mL rapamycin. After 21 days the cells expanded 200-fold but although still enriched for CD4+Foxp3+CD27+ cells compared cultures grown without rapamycin, the suppressor activity and Foxp3 content had greatly declined. We have further optimized conditions by exploring two approaches: 1) increase the starting purity of CD4+Foxp3+ cells, and 2) increase the dose of rapamycin to further inhibit the expansion of activated T cells. We compared the purity of Tregs when 1, 2, or the previously used 4 μL of CD25-coated beads/10^7 starting cells were used to bind the CD25+ cells and 1 versus 2 selection columns. Data showed that after passage through one column the % of CD4+ cells expressing Foxp3 was 27.3%, 54.3% and 31.7% with 1, 2, or 4 μL beads, respectively. The %CD4+Foxp3+ cells further increased to 51.9%, 64.1% and 60%, respectively after a passage through a second column. Therefore, subsequent experiments used 2 μL beads and sequential passage through two columns. Doses of rapamycin from 50-200 ng/mL were increasing toxic, with few cells recovered from the expansion cultures. However, at the previously used doses of 5-10 ng/mL the %CD4+Foxp3+ cells after 22-24 days averaged 37.3±7.1%, similar to the 42±20% seen at day 10 in our previous experiments and significantly higher than the 23±2% previously seen at day 21 when suppressor activity had declined. In contrast only 4.7±0.4% of the recovered CD4+ cells expressed Foxp3 in the absence of rapamycin, similar to the 5±3% shown for single