When used as therapy for hematopoietic malignancies, allogeneic bone marrow transplantation (BMT) relies on the graft-versus-leukemia (GVL) effect to eradicate residual tumor cells through immunologic mechanisms. However, graft-versus-host-disease (GVHD) is a potentially lethal complication of allogeneic BMT. Thus, inhibition of GVHD, while preserving GVL and protective responses against infectious agents, can enhance the therapeutic potential of BMT. GVHD is initiated by alloreactive donor T cells that recognize mismatched major (MHC) and/or minor (MiHA) histocompatibility antigens and cause severe damage to hematopoietic and epithelial tissues. Protein kinase C theta (PKCθ) is a key regulator of T cell-receptor signaling. We show here that T cell responses triggered by Listeria or following administration of antigen plus microbial adjuvant are relatively well preserved in the absence of PKCθ. In contrast, we demonstrate an essential requirement for PKCθ in alloreactivity and GVHD induction. Furthermore, absence of PKCθ raises the threshold for T cell activation, which selectively impacts alloseponses. Most importantly, PKCθ−/− T cells retain both the ability to respond to virus infection and induce GVL post BMT. These findings validate PKCθ as a potentially unique therapeutic target that is required for GVHD induction but not for GVL or protective responses to infectious agents.

425 FEASIBILITY OF NIH CONSENSUS CRITERIA FOR CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD): SUCCESSFUL EFFORTS IN ESTABLISHING A MULTICENTER BRAZIL-SEATTLE COLLABORATIVE CONSORTIUM

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Advances in clinical trials for treatment of chronic GVHD have been hampered by slow accrual of patients (pts) and by inconsistency and limitation of historical systems for grading used prior to the 2005 NIH consensus criteria for diagnosis and classification of chronic GVHD (Filipovich AH et al BBMT 2005;11:945-956). In an effort to foster collaboration and expand the network for future clinical trials in chronic GVHD, a Brazil-Seattle consortium was established in 2008 to conduct chronic GVHD studies in Brazil. The Brazil-Seattle chronic GVHD consortium included 15 multidisciplinary participants (i.e., hematopoietic cell transplant (HCT) physicians, dermatologist, dentist, psychiatrist, psychologist, physiotherapist and one data manager) from 5 Brazilian institutions and two experts in chronic GVHD from Seattle. The consortium held monthly teleconferences and met at least twice yearly during national and international HCT and Hematology meetings in Brazil and USA. After training and translation of the NIH consensus criteria tools into Portuguese, the group initiated a feasibility study using the NIH consensus criteria after approval by each of the 5 participating centers' institutional review boards. So far, 34 pts with NIH chronic GVHD after allogeneic HCT in Brazil have been enrolled between June 2006 and May 2009 and prospectively followed. Of the 34 pts with NIH chronic GVHD, 26 (76%) met the overlap syndrome subcategory and 8 (24%) the classic subcategory. The overall severity of NIH chronic GVHD was moderate in 21 pts (62%) and severe in 13 pts (38%). The median time from HCT to onset of NIH chronic GVHD overlap syndrome subcategory was 5.9 months (2.5-10) and from transplant to NIH chronic GVHD classic subcategory was 7.3 months (3.0-16). At a median follow up of 16.5 months, the overall survival was 70%. At least 5 pts were enrolled from each center. In conclusion we demonstrate the feasibility of the NIH consensus criteria for the diagnosis and scoring of chronic GVHD in a prospective Brazilian multicenter study and, more importantly, in establishing a coordinated HCT network in Brazil that offers new opportunities for future intervention, validation, and biomarkers clinical trials in chronic GVHD.

426 INCIDENCE OF GRAFT-VERSUS-HOST DISEASE (GVHD) IN PATIENTS WITH ALLOGENEIC PERIPHERAL HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER A NON-MYELOABLATIVE CONDITIONING

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Introduction: Graft-versus-host disease (GVHD) is the most common complication of allogeneic hematopoietic cell transplantation (alloHCT) and may affect the transplant outcome. Its incidence is higher when the preparative regimen used is a non-myeoablative and the stem cell’s source is peripheral blood after mobilization.

Objective: To demonstrate the 10 years incidence of GVHD in 2 Mexican transplant centers using peripheral hematopoietic stem cell transplantation (PHSCT) in related donors after non-myeloablative conditioning.

Patients and methods: Three hundred and fifty six patients with hematological and non-hematological malignancies that underwent PHSCT after non-myeloablative conditioning between October 1998 and November 2008 were included. The age ranged between 1 and 71 years (median of 29). Two hundred and nineteen patients were men and 137 women. They received cyclosporine 4 mg/kg per day and intramuscular methotrexate 5 mg/m2 in days +1, +3, +5 and +11 for GVHD prophylaxis.

Results: Three hundred and fourteen (88%) patients were successfully engrafted. One hundred and fifty six patients (49%) developed acute and/or chronic GVHD. Of this last number of patients, 68 (44%) developed acute GVHD, 44 (28%) developed chronic GVHD, and 44 (28%) developed both. Twenty three percent (26) of the patients who developed acute GVHD were grade III or IV, and 35% (31) of chronic GVHD patients presented in the extensive way. GVHD was the cause of death in 33 patients (12%), even when immunosuppressive therapy (high dose steroids and rituximab) was used. Last death was 24 months ago; subsequently, alemtuzumab was included in GVHD treatment.

Conclusion: The incidence of acute and chronic GVHD in our patients is lower than the reported in the literature, even though the source of hematopoietic stem cells was peripheral blood. The mortality rate has decreased due to the introduction of new are more aggressive immunosuppressive agents like alemtuzumab.

427 MOBILIZING CELLS WITH A TOLEROGENIC PHENOTYPE USING PEGYLATED RECOMBINANT MURINE GM-CSF: IN VITRO AND IN VIVO EFFECTS

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Granulocyte-macrophage colony stimulating factor (GM-CSF) is a growth factor regulating proliferation and differentiation of hematopoietic stem and progenitor cells. Murine GM-CSF (mGM-CSF) has a very short half-life and pegylation of mGM-CSF (peg-mGM-CSF) has been shown to prolong its half-life. Based on our clinical observations that patients transplanted with stem cell products mobilized by GM-CSF have a lower incidence of acute GVHD, we hypothesized that peg-mGM-CSF may be protective of acute GVHD in a HMA mismatched murine model.

We have shown that C57/Bl6 mice treated with peg-mGM-CSF led to increased CFU-C by 26.5 fold compared to PBS treated mice (n = 6, 95% CI = 10.25-42.73). (ASH 2009) In addition, we observed that peg-mGM-CSF increased CD4+FoxP3+Tregs in both blood and spleen by 2 fold. (p < 0.05) (ASH 2009) This effect was sustained out to 4 days after the last dose of peg-mGM-CSF. Importantly, the function of these peg-mGM-CSF Tregs, as measured in a MLR was found to be equivalent to Tregs from PBS treated control mice (n = 3, p = 0.27) (ASH 2009)

To assess the in vivo impact of peg-mGM-CSF on graft versus host disease, we used the C57/Bl6 → Balf/c acute GVHD model.
We hypothesized that treatment of recipients peri-transplant with peg-mG-CSF may be protective of GvHD and improve survival by increasing recipient Tregs. Balb/c mice (n = 5/group) were treated with peg-mG-CSF or PBS for 4 days prior to transplantation using myeloablative conditioning and transfer of B6 splenic T-cells and bone marrow. This was followed by continued treatment with either PBS or peg-mG-CSF for the first week after transplant. There was a trend toward worse survival in the peg-mG-CSF treated recipients. (Log-rank [mantel-cox] test, p = 0.085) We next asked if treatment of T-cell donors might abrogate GvHD. B6 donors were treated with G-CSF, peg-mG-CSF or PBS for 4 days, on the fifth day splenocytes were isolated and pan-T-cell selected using an AutoMACS column. An equivalent dose of CD3+ T-cells along with congenic bone marrow was administered to recipient Balb/c mice (n = 4-5/group) that had undergone myeloablative conditioning. Mice receiving GM and G treated donor T-cells had an improved survival compared to PBS alone (PBS vs. G p = 0.0314, PBS vs. GM p = 0.08467 (Log rank [Mantel-cox test]) but displayed similar GvHD scores. This effect may be explained by an increase in Tregs delivered in the G and GM group. Further studies will seek to validate these results.

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CD4+ T CELLS ACCUMULATE IN THE COLON OF CSA-TREATED MICE FOLLOWING MYELOABLATIVE CONDITIONING AND SYNGENIC BONE MARROW TRANSPLANTATION

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Syngenic graft-versus-host disease (SGVHD) was first described as a GvHD-like syndrome that developed following syngenic BMT and cyclosporine A (CsA) treatment. SGVHD is induced by reconstituting lethally irradiated mice with syngenic bone marrow cells followed by a 21 day treatment with the immunosuppressive agent CsA. Clinical manifestations of the disease appear 2 to 3 weeks following cessation of CsA therapy and disease-associated inflammation occurs primarily in the colon and liver. CD4+ T cells have been shown to play an important role in the inflammatory response observed in the gut of SGVHD mice. Time course studies revealed a significant increase in the migration of CD4+ T cells into the colon during CsA therapy (as early as day 14 post-BMT). Significantly elevated levels of proinflammatory cytokines, chemokines and cellular adhesion molecules were observed in the colonic tissue of CsA treated animals compared to BMT controls. Homing studies revealed that a labeled CD4+ T cell line, generated from SGVHD mice, migrated to a greater extent into the gut of CsA treated mice at day 21 post-BMT as compared to control animals. The migration of CD4+ T cells during the 21 days of immunosuppressive therapy, functional mechanisms are in place that result in increased homing of CD4+ T effector cells to the colons of CsA-treated mice.

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ATYPICAL PRESENTATION OF VAGINAL GvHD WITH ABDOMINAL PAIN CRISIS

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Rare causes of abdominal pain may be overlooked in allogeneic transplant recipients with recurrent episodes of abdominal pain. We describe a 19 year-old female, who received a matched unrelated donor peripheral blood stem cell transplant for acute myeloid leukemia-M4, presenting with recurrent abdominal pain. The transplant conditioning regimen was fludarabine (75 mg/m2) and melphalan (140 mg/m2) and graft versus host disease (GvHD) prophylaxis was tacrolimus and methotrexate. The post transplant course was complicated by acute and chronic GvHD of the GI tract and skin, treated with calcineurin inhibitors, glucocorticoids, mycophenolate mofetil, sirolimus, azathioprine, extracorporeal photopheresis and narrow beam ultraviolet B (UVB). 18 months after transplantation, she presented with recurrent crises of abdominal pain. Endoscopic biopsies were negative for GI GvHD. CT scan and ultrasound of the abdomen suggested a complex 6 × 9 cm adnexal cyst arising from the left side. Diagnostic laparoscopy findings were inconsistent with a cyst. A subsequent CT of the pelvis suggested a hematocoele. Vaginal exploration identified a transverse vaginal septum occluding menstrual flow. Transsection was performed and a copious amount of blood was drained with relief of symptoms. Patient presented two months after surgery with recurrent pain and ultrasonogram demonstrated hematocoeles requiring resection of the vaginal septum to alleviate the obstruction. Patient’s post operative course was complicated with tubo-ovarian abscess resulting with rupture and septic shock; she underwent laparoscopic right salpingo-oophorectomy. The patient improved and was released from the hospital 2 weeks later. Biopsy of the vaginal obstruction showed a segment superficially ulcerated endocervical-type columnar mucosa and portions of fibromuscular tissue consistent with vaginal GvHD. In summary, scarring from unrecognized vaginal GvHD led to retention of menses and chronic abdominal pain. She is now using vaginal dilators with topical steroids to prevent further recurrence and clinically doing well. This case is presented to highlight awareness of this rare complication.

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NEPHROTIC SYNDROME AFTER HEMATOPOIETIC CELL TRANSPLANTATION: MANIFESTATION OF GvHD

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Although severe nephrotic syndrome (NS) has been found as a major symptom of cGVHD in murine models, the effect of GvHD on human kidney has not been studied extensively. We describe a case of NS in 16 years old female patient that was submitted to an identical sibling allo BMHSCT for CML in 1st CP. The conditioning was the BuCy regime. She received 3.8 × 109 mononuclear cells/kg. The GvHD prophylaxis was CSA/MTX. On D + 180, she was Phi and BCR-ABL negative and free from immunosupression without any symptoms of GvHD. Five years after transplantation, she presented a cytogenic relapse with 20% of Phi+ cells. She was treated with two DLI infusions (1.5 × 107 CD3+ cells/kg and 1 × 107 CD3+ cells/kg) and she became BCR-ABL negative. Until two years after the second DLI she never presented any signs or symptoms of GvHD. After that, she started with intermittent urticaria and edema on the face that progress to anasarca. The clinical and laboratory investigation revealed proteinuria 2 to 8 g/24 h, hypoalbuminemia (2.3 mg/dl) and hematuria. Phi chromosome, BCR-ABL, serology for virus, syphilis and colagenosis antibodies were all negative. A kidney biopsy revealed findings of early membranous glomerulonephritis with mild mesangial proliferative glomerulopathy. The IFM confirmed granular immune deposition for IgG along the capillary loops. She was initially treated with prednisone 1 mg/kg plus a loop diuretic and an angiotensin II receptor blocker. Secondary to steroid resistance, cyclophosphamide 1 g/m2 plus rituximab 325 mg/m2 for two times, in 15 days interval, was administered. Six weeks after the second cycle of Cy/Rituximab the intermittent urticaria and 8 g/24 h proteinuria were still present. Thus, the patient started CSA 5 mg/kg. After six months of CSA, the urticaria and proteinuria (170 mg/24 h) disappeared completely, and albumin reaches 3.6 mg/dl. Now, she is in a tapering regimen for CSA. NS following HSCT is a rare complication. It is mainly related to membranous nephropathy, that is frequently to minimal change disease, focal segmental glomerulonephritis, diffuse proliferative glomerulonephritis or IgA nephropathy. Other renal complications of HSCT include drug and radiation toxicity and thrombotic microangiopathy that need to be ruled out in the differential diagnosis. In conclusion: the literature supports the existence of renal GvHD. However, further investigations are warrant since the pathophysiology is unclear and there is no treatment established.