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HIGH RATE OF *DE NOVO* CHRONIC GRAFT-VERSUS-HOST (CGVHD) FOLLOWING BUSULFAN-FLUDARABINE CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION FROM A MATCHED-SIBLING DONOR (MSD) FOR AML/MDS

Saliba, R.M.[1], Komanduri, K.V.[2], Koca, E.[1], Alousi, A.M.[1], Giralt, S.[1], Andersson, B.S.[1], Zhang, W.[1], Rondon, G.[1], Neumann, J.[1], Popat, U.R.[1], Kebriaei, P.[1], de Lima, M.[1], Champlin, R.E.[1] [1] University of Texas MD Anderson Cancer Center, Houston, TX; [2] University of Miami Sylvester Cancer Center, Miami, FL

Busulfan-fludarabine is increasingly used as a conditioning regimen for allogeneic stem cell transplantation (ASCT). Fludarabine has complex immunologic effects which may impact the development of GVHD. Risk factors and outcomes of cGVHD in this setting have not been determined. We retrospectively evaluated all consecutive patients (pts) who received conditioning with IV Busulfan (130 mg/m^2 for 4 days) and Fludarabine (40 mg/m^2 for 4 days) and ASCT from a MSD at MD Anderson Cancer Center between 2001 and 2005. In a landmark analysis starting at day +100, we estimated the cumulative incidence (CI) of cGVHD (defined according to the recent NIH consensus diagnostic criteria); evaluated risk factors for overall and *de novo* cGVHD using ^{Cox}'s regression analysis; and evaluated the effect of cGVHD on outcome.104 pts were included in this analysis. Median age was 46 years (12–65). 46% were females and 49% were in complete remission at the time of ASCT. Peripheral blood (PB) was the stem cell source in 85% of pts. GVHD prophylaxis consisted of tacrolimus and methotrexate at standard doses. The incidence of grade II-IV aGVHD was 18%. 89 pts were alive on day +100 without progression of their malignancy including 51 pts who had not developed aGVHD. With a median follow-up of 46 months in survivors, 57/89 pts developed cGVHD; half (n = 29) of these cases occurred *de novo.* The 3 years CI of *de novo* cGVHD was 57% in the 51 pts without antecedent aGVHD. Time of onset was later for *de novo* cGVHD compared with relapsing/progressive cGVHD; median 195 days (115–693) vs. 143 days (100–639). The use of PB was the only significant risk factor for development of cGVHD ($HR_{overall}$ = 3.3, *P* 0.01; $HR_{de\ novo}$ = 5.0, *P* 0.03). Recipient age, recipient/donor sex and CMV serostatus, and disease status prior to ASCT had no significant impact on the rate of cGVHD. On univariate analysis considering cGVHD as time-dependent variable, there was a trend toward an association of *de novo* cGVHD, but not relapsing/progressive cGVHD, with a lower rate of disease progression (HR = 0.6, *P* 0.4) and mortality (HR = 0.4, *P* 0.1). There was also a trend for more favorable overall survival (HR = 0.4, *P* 0.08) and non-relapse mortality (HR = 0.3, *P* 0.2) in pts who developed *de novo* compared with relapsing/progressive cGVHD. There is a high frequency of *de novo* cGVHD after busulfan-fludarabine conditioning and ASCT for AML/MDS. This suggests a shift in the epidemiology of cGVHD that merits further consideration.

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EFFICIENT AND SELECTIVE PREVENTION OF GRAFT-VERSUS-HOST DISEASE BY ANTIGEN-SPECIFIC TGF-INDUCED REGULATORY T CELLS

Semple, K.[1,3], Iclozan, C.[1], Anasetti, C.[1,2], Yu, X.-Z.[1,2,3] [1] H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; [2] University of South Florida, Tampa, FL; [3] University of South Florida, Tampa, FL

Naturally occurring regulatory T cells (nTregs) suppress the development of graft-versus-host disease and may spare graft-versus-tumor effect. As nTreg is a rare cell population in a healthy individual, using in vitro expanded nTregs is a common strategy to test their therapeutic potential in hematopoietic cell transplantation (HCT). However, the concern of in vitro expanded nTregs may include their stability of Foxp3 expression and suppressive function, survival in vivo, and non-selective suppression of pre-activated nTregs. In this study, we have used an alternative strategy to generate antigen-specific, induced Tregs (iTregs). CD4+CD25- cells from OT-II TCR transgenic, foxp3/gfp knock-in mice were induced to express Foxp3 by incubating with OVA peptide in the presence of TGFβ. CD4+GFP+ cells were purified by sorting and used as iTregs while CD4+GFP- cells as controls. Their ability to prevent GVHD was tested in a lethally irradiated murine BMT model: B6 → (B6 x bm12)F1. In order to evaluate the specificity of iTregs, OVA-expressing or non-expressing F1 recipients were directly compared. We found that iTregs (CD4+GFP+) efficiently prevented GVHD lethality in OVA+ recipients at a Treg:Teff ratio up to 1:8. The efficacy of these antigen-specific iTregs were significantly higher than polyclonal iTregs from B6 donors as the latter could only partially prevent GVHD and prolong recipient survival even at a 1:1 ratio. In contrast to OVA+ recipients, antigen-specific iTregs failed to prevent GVHD in OVA- recipients. As controls, CD4+GFP- cells had no effect on GVHD development in OVA- recipients, and even exacerbated GVHD in OVA+ recipients compared to B6 CD4+ effector cells alone. These results reveal the therapeutic potential of antigen-specific iTregs to prevent GVHD efficiently and selectively.

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EXPLORING T-CELL RECRUITMENT DURING THE EARLIER PHASE OF GRAFT VS. HOST DISEASE *IN VITRO*

Arditti, F.D., Goldgirsh, K., Friedman, T., Korngold, R. Hackensack University Medical Cente, Hackensack, NJ

Graft-versus-host disease (GVHD) is the major complication following allogeneic blood and marrow transplantation (BMT). Although major advances have been achieved in understanding the immunopathology of GVHD, the rate of success in the clinic has been hampered by the inability to improve overall survival. T cell-depletion of the hematopoietic stem cell inoculum has been proved efficient in preventing GVHD, however, this treatment abolished the graft-versus-leukemia (GVL) effect, also mediated by donor T cells, resulting in significant increases in the rates of relapse. Therefore, other means for separating GVHD and GVL need to be considered. Activation and recruitment of T cells to targeted organs during GVHD is preceded by a well orchestrated, but yet unknown in depth, sequence of events that involves the early secretion of chemokines produced by a wide variety of cells, including dendritic cells, mast cells, T cells and their primary targets during GVHD, epithelial cells, in the skin and the gastrointestinal tract. In this study, we take advantage of our recently developed *in vitro* model of allogeneic T cell/epithelial interactions to investigate this less explored aspect of GVHD. Using a high throughput multicytokine assay, we examined the secretion of different chemokines in cocultures of skin primary epithelial cells (pEC), obtained from C.B10-H2b (BALB.B) mice, with allogeneic T cells, derived from minor histocompatibility antigen allogeneic C57Bl/6 (B6) mice. In addition to IFN-γ and IL-12, we detected the presence of MCP-1/CCL2, MIP-1α/CCL3, RANTES/CCL5 and GM-CSF by 7 days after the addition of the allogeneic T cells. Although we do not know yet in detail whether T cells, epithelial cells, or both are responsible for the secretion of the chemokines in our system, their level was dependent on the amount of T cells added to the cocultures ($p<0.03$). However, we found that in the absence of T cells, conditioning of the pEC by exposure to 11 Gy of γ-irradiation induced the secretion of 109.6 pg/ml MCP-1 and 21.7 pg/ml RANTES within 24 hours. Thus, our preliminary data suggests that the conditioning of epithelial cells, alone, is enough to induce secretion of chemokines, which in turn could induce T cell recruitment to the site before any damage to the epithelial cells can be visualized, leading subsequently to an amplification cascade governed by chemokine secretion from both target and effector cells.

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WHAT IS THE ROLE FOR REGULATORY T-CELLS AFTER NONMYELOABLATIVE CONDITIONING?

Humblet-Baron, S., Castermans, E., Vanbelleghen, J.-F., Hannon, M., Jacobs, N., Willems, E., Ormenese, S., Beguin, Y., Baron, F. University of Liège, Liège, Belgium

Purpose: We investigated the association between regulatory T-cell (Treg) levels and chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HCT) following nonmyeloablative conditioning.

Methods: Data from 74 patients given nonmyeloablative conditioning as treatment for hematological malignancies or renal cell carcinomas were analyzed. Conditioning regimens consisted of