(29%) developed a thrombotic thrombocytopenic purpura/microangiopathic hemolytic anemia syndrome or an acute hemolytic reaction that was treated with plasmapheresis or red cell exchange. Twenty-one of 30 (70%) evaluable patients developed grade II-IV acute GVHD. Of these, 62% had grade III-IV acute GVHD. Sixteen of 21 (76%) evaluable patients, developed chronic GVHD with 63% of those patients having extensive chronic GVHD. The day 100 survival was 68%. Ten patients are alive at a median of 67.6 months (range: 45.2-82.3), for an overall survival of 32%. These results indicate that, in contrast to its effects after allogeneic BMT, single-agent tacrolimus does not adequately prevent GVHD after related allogeneic PBSCT.

110

PHASE II STUDY OF DENILEUKIN DIFTITOX (ONTAK) IN THE TREATEMENT OF STEROID RESISTANT ACUTE GRAFT VERSUS HOST DISEASE (AGVHD)

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AGVHD is one of the pricipal complications of allogeneic hematopoietic stem cell transplantation. Ontak is a recombinant DNA derived cytotoxic fusion protein designed to direct the cytocidal action of diphtheria toxin to cells with high affinity of the IL-2 receptor. We designed a phase II trial to examine the Ontak in patients (pts) with steroid resistant AGVHD. Steroid resistance was defined as treatment with 2mg/kg of methylprednisolone and progressive disease after 3 days or stable disease after 7 days. Ontak treatment was 4.5mcg/kg/day IV for 5 days followed by the same dose weekly for 4 weeks. Four pts have been treated to date. Pt #1 had stage 3 skin and stage 4 GI AGVHD that improved to stage 1 skin and stage 1 GI by day 5 of the Ontak. Ontak was held in this pt because of a low albumin and fluid retention. GVHD recurred 4 weeks after Ontak was held and, despite treatment with inflixamab, GVHD progressed and the pt died of psuedomonas sepsis. Pt #2 had stage 3 skin AGVHD that responded completely by day 5 of the Ontak and finished 3 of the 4 weekly treatments, with the last treatment held for pancytopenia. Pt #3 had stage 4 GI and conjuctival AGVHD that completely resolved by day 5 of Ontak and finished all treatments. Pt #4 had stage 4 liver, 3 skin, and 4 GI AGVHD that progressed after the first 5 day infusion of Ontak and was taken off study and treated with infliximab with an improvement to stage 3 liver, 0 skin, and 4 GI AGVHD. Pt #4 had a generalized tonic-clonic seizure on day 2 of the Ontak infusion that was felt to be related to cyclosporine and completed the Ontak infusion with no further neurologic sequalae. Pt #4 also developed disseminated adenovirus. Pts #2 and #3 had grade III hematologic toxicity that was felt to be multifactorial and both also had bacterial upper respiratory tract infections that were treated and resolved. Patient #3 had CMV reactivation that was pre-emptively treated with valgancyclovir and resolved. Pts #2 and #3 are both greater than 100 days from the start of the Ontak treatment and have been tapered to less than 0.5mg/kg or steroids while remaining on FK506 with no evidence of AGVHD. In conclusion, the low dose of Ontak used in this study appears active in AGVHD with acceptable toxicity. Further study to assess infection risk, response rates, and patient survival are warranted.

Patient	Age	Disease	Donor	GVHD prophylaxis	Max GVHD grade	GVHD grade post Ontak	Current Status
1	36	NHL	matched related	CYA/MTX	IV	II	Died day 100 progressive NHL/sepsis Grade IV GVHD
2	33	CML	matched unrelated	FK506/MTX	II	0	Alive day 160 in CR No GVHD
3	58	NHL	matched related	FK506/MTX	IV	0	Alive day 130 in CRu No GVHD
4	9	AML	mismatched unrelated	CYA/steoids	IV	IV	Alive day 100 in CR grade III GVHD

111

SELECTIVE DEPLETION OF HUMAN GVH-REACTIVE T-CELLS BY ANTI-CD95 MEDIATED ACTIVATION-INDUCED CELL DEATH OR VIA THE T-CELL ACTIVATION ANTIGEN CD69 FOR PREVENTING GRAFT-VERSUS-HOST DISEASE IN ALLO-BMT

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Depletion of T-cells from allogeneic bone marrow transplants (BMT) ameliorates GvHD but is associated with impaired engraftment, immunosuppression, and abrogation of the beneficial graft-versus-leukemia effect (GvL). We, therefore, explored the possibility of separating alloreactivity from T-lymphocytes mediating GvL-responses *ex vivo* by selective depletion of GvH-reactive T-cells using CD95/CD178-mediated activation-induced cell death (AICD) or via the T-cell activation antigen CD69. T-cells purified from human PBMC were stimulated with fully-mismatched stimulators in an allogeneic mixed lymphocyte culture (MLC) in the presence of agonistic anti-CD95 mAb. Specificity of depletion was tested by consecutively monitoring CMV-pp65 specific T-cells present in PBMC from seropositive donors before and after AICD. As reported earlier in a murine model, proliferative responses of alloreactive T-cells were reduced 65-90% in the presence of anti-CD95 reagents depending on the mAb used. ELISpot analysis revealed the retainment of pp65-specific T-cells in the residual allogeneic T-lymphocyte population comparable to frequencies detected in normal controls. In comparative studies, elimination of alloreactive specificities by immunomagnetic separation using the T-cell activation marker CD69 resulted in 3 subsets of T-cells. Restimulation of $CD69^-$ cells ($\geq 98\%$) as well as the $CD69^+$ population and the $CD69^{dull}$ cells retrieved from the wash-fraction with 1st party stimulators resulted in a substantial reduced proliferation of the $CD69^-$ cells as compared to the positive subset. The $CD69^{dull}$ T-lymphocytes also showed residual alloreactivity. Interestingly, monitoring for pp65-reactive T-cells by ELISpot analysis indicated that the majority of pp65-specific T-cells appeared to be enriched in the $CD69^{dull}$ population. Further studies on the efficacy, specificity and potential of retained allogeneic T-cells to respond to known leukemia-associated antigens in HLA-mismatched as well as HLA-matched settings are in progress and will be discussed.

112

SERUM CYTOKINE PROFILES OF PATIENTS WITH ACUTE GVHD TREATED WITH METHYLPREDNISOLONE ALONE OR IN COMBINATION WITH INFLIXIMAB ARE DIFFERENT

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Graft-versus-host disease (GVHD) is a major problem for recipients of allogeneic blood and marrow transplantation. GVHD, mediated by TNF-alpha produced by activated mononuclear cells, can be treated with methylprednisolone (MP) or in combination with Infliximab (I), a chimeric human/mouse anti-TNF antibody that interferes with the activity of TNF-alpha. Moreover, as acute GVHD (aGVHD) is linked to increases in the levels of inflammatory cytokines, we hypothesized that there would be a decrease in inflammatory cytokines as patients achieved a complete response following treatment for aGVHD. Therefore, we collected sera from 52 patients with aGVHD who were randomized to one of two treatment arms to receive either methylprednisolone (MP) or MP plus Infliximab (MP+I) once a week for 4 weeks. We measured serum levels of IL-2, IL-6, IL-10, IL-12, TNF-alpha, and IFN-gamma pre-treatment and at weekly intervals thereafter for 5 weeks on a subset of patients with aGVHD. We analyzed cytokine data for 10 MP and 10 MP+I patients. The levels of IL-6, IL-10, IL-12, and IFN-gamma before and after treatment were similar for both groups. The profiles of IL-2 and TNF-alpha levels over time however were different for the 2 groups. The levels of these cytokines in the MP+I group peaked in the first two weeks of treatment and declined thereafter. Conversely, the levels of IL-2 and TNF-alpha in the MP group were low in the first two weeks of treatment and increased thereafter. These preliminary data suggest that there may be differences in the profiles of IL-2 and