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Allogeneic hematopoietic stem cell transplantation (HSCT) is commonly used as a treatment for hematological malignancies, immunodeficiencies, and inborn errors of metabolism. HSCT is associated with several complications and risk factors, for example graft-versus-host disease (GVHD), viral infections, relapse, and graft rejection. Infusions of extra lymphocytes from the original stem cell donor can be used as a treatment after transplantation for, e.g., relapse, poor immune reconstitution or secondary graft failure. The term booster applies for infusions of donor cells without conditioning. Secondary graft failure was in this study defined as initial signs of engraftment with subsequent development of bone marrow hyperplasia requiring frequent transfusions beyond day 60 as well as prolonged neutropenia and thrombocytopenia. While high levels of CD3+ cells in grafts can increase the risk for GVHD they can also promote the graft versus leukemia (GVL) effect. In peripheral blood, 95% of T-cells express the αβ T-cell receptor and the remaining T-cells express the γδ T-cell receptor, which are not dependent upon human leukocyte antigen (HLA) molecules for activation. As αβ T-cells are the primary mediators of GVHD, depleting them from the graft should reduce this risk, leaving γδ T-cells as primary T–cell type in the booster infusion. In this pilot study, five patients transplanted with HLA-matched related and unrelated donors were treated with αβ T-cell depleted stem cell boosts, with secondary graft failure as primary indication. All patients also suffered from infectious complications prior to the infusion, which is likely to have been associated with the poor graft function and development of secondary graft failure. The αβ T-cell depletion was performed using a CliniMACS system (Miltenyi Biotech). Analysis of the booster infusion fraction as well as blood samples from the patients was performed; cell characterization by using flow cytometry and evaluation of chimerism as well spectratyping of γδ-chains by using PCR. Monitoring of white blood cells, platelets, and granulocytes was carried out for 30 days post infusion. The majority of γδ T-cells in the grafts expressed Vδ2 and/or Vδ9. Median log depletion of γδ T-cells in the products was 3.72, and the mean yield of γδ T–cells was 84.4%. Most patients receiving αβ-depleted stem cell boosts increased their levels of white blood cells, platelets, and/or granulocytes 30 days post infusion. No signs of GVHD or other side effects were detected. A larger pool of patients with longer follow-up time is needed to confirm the data in this study and compare the treatment modality to other therapy options.

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Hypomethylating Agents Induce FOXP3 Negative HLA-G Expressing Immunoregulatory T Cells

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A major caveat of FOXP3+ T regulatory cells in immunotherapy against GVHD is their low numbers in circulation and the lack of specific cell surface markers for efficient purification. DNA methylation plays a key role in the regulation of T-cell effector function and cytokine gene expression, indicating a promising role of hypomethylating agents in immunomodulation. Recently it was shown that in vitro treatment of conventional T-cells with the hypomethylating agent azacitidine (aza) induced FoxP3 expression and converted $CD4^+CD25^-$ cells into immunosuppressive T-cells, the suppressor function of which is independent of FoxP3 expression (Blood 2010;116:129-139), suggesting that aza induced suppressor function depends on the modification of other hypomethylated genes. Human leukocyte antigen-G (HLA-G) is a non-classical HLA class I molecule, shown to exert immunoregulatory functions, the expression of which is epigenetically regulated. In this study we investigated whether hypomethylating agents (HAs) can induce $HLA\text{-}G^+$ immunoregulatory T cells. Negative selected T cells from peripheral blood of healthy individuals were stimulated with anti-CD3 plus anti-CD28 coated beads, then treated for 72 hours with aza (0.5-15 mM) in the presence of 50U/ml inteleukin-2 (IL-2). Phenotypical characterization of aza treated cells was performed with flow cytometry. The effect of HAs on HLA-G mRNA transcription and methylation of upstream regulatory HLA-G gene DNA sequence was quantitated with Real time PCR and bisulfite pyrosequencing respectively. Hypomethylation-induced $HLA\text{-}G^+$ T cells, were irradiated and then used as third party cells in suppression assays. In vitro treatment of $CD3^+$ T cells with aza induces, dose dependent, de-novo HLA-G expression on stimulated peripheral T cells of healthy individuals. The optimum aza concentration for maximum HLA-G induction with the lowest toxicity in CD3 T cells was determined at 5 mM (surface expressing HLA-G cells: 6,88±3,9%, p=0,0022, fold increase in HLA-G mRNA relevant expression: 18,32±2,33). Methylation analysis of 13CpGs revealed that treatment with HAs caused hypomethylation of the HLA-G upstream-regulatory region followed by mRNA transcription and up-regulation of surface protein expression. Stability assays revealed that HLA-G expression is evident for at least 3 days after removal of hypomethylating agent. In vitro Aza induced $HLA\text{-}G^+$ cells are immunosuppressive and HLA-G blocking experiments further confirmed that their suppressive function is largely but not exclusively dependent on HLA-G. Phenotypic analysis revealed that aza induced $CD4^+HLA\text{-}G^+$ are FoxP3 negative $CD4^{low}CD25^{high}$. Conclusively, by using hypomethylating agent aza in vitro, we generated a $CD4^{low}FoxP3^{neg}$ immunoregulatory population, which expresses extracellular HLA-G and therefore can be easily isolated for adaptive immunotherapies.

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Novel Strategy to Enhance NK Cell Activity

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Novel therapeutic approaches are urgently needed for many malignancies such as Acute Myeloid Leukemia (AML). We have developed a new therapeutic strategy based upon NK cell immunotherapy that exhibits high clinical potential based upon cell and animal studies. While the harnessing of NK cells for cellular therapy against malignancies has been a topic of interest for several decades, our approach overcomes a major hurdle of insufficient NK cell cytotoxic activity. We have identified that targeting the kinase GSK3 through pharmacologic and genetic approaches leads to the hyperactivation of human blood derived NK cells and a significant