observed in 3 patients (25%). There was no response in 2 patients (16%). The overall response rate at 4 weeks was 83%. Infectious complications were common, including bacteremia (41%), adenovirus viremia (50%), and CMV viremia (33%). Relapse of acute GVHD was observed in 42% of complete responders. Fifty percent of patients are currently surviving at a median follow up time of 775 days following first alemtuzumab course. We conclude that alemtuzumab is an effective treatment for steroid refractory GVHD in pediatric patients with a tolerable spectrum of complications. The dose, timing, and length of treatment should be optimized in a prospective study.

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THERAPY OF STEROID-REFRACTORY CHRONIC GVHD WITH SUBCUTANEOUS LOW-DOSE ALEMTUZUMAB AND RITUXIMAB COMBINATION IS EFFECTIVE AND SAFE

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Introduction: Chronic graft-versus-host disease (cGVHD) is a common late complication of allogeneic hematopoietic stem cell transplantation. Corticosteroids with or without a calcineurin inhibitor are the standard treatment for cGVHD. A second-line treatment is not well defined. A simultaneous reduction of both B and T-cells might be an efficacious treatment strategy for cGVHD, gaining a strict control of the immune system responsible of pathophisiology of cGVHD. We evaluated the effectiveness and safety of low doses of alemtuzumab and rituximab as treatment for steroid-refractory cGVHD.

Methods: Ten men and 5 women were included. All patients had received an identical-HLA allo-RIC peripheral stem cell transplant and had cGVHD refractory to steroids and CSA according to NIH criteria's. The median age was 41 years old. The main site involved was the oral mucosa (87%) followed by the eyes (67%), liver (60%), skin (53%), lungs (13%) and intestinal tract (7%). All patients received subcutaneous Alemtuzumab 10 mg on days +1 to +3 and intravenous Rituximab 100 mg on days +4, +11, +18 and +25.

The therapeutic response was measured on days +30, +90 and +365 of the protocol.

Results: The overall response was 100% at +30-day evaluation, 10 patients had partial remission (PR) and 5 patients had complete remission (CR). At +90-day evaluation, 7 patients had PR, 4 CR, 3 had cGVHD relapsed and 1 patient had not yet reached 90 days. Currently, 5 patients have reached the +365-day follow-up evaluation, 2 (40%) had PR, 2 had CR and 1 showed cGVHD progression. The adverse effects were mainly infection in 60% of patients, quickly solved in all cases, except one patient who died from pneumonia.

Conclusion: Low dose alemtuzumab-rituximab combination therapy appears to be an efficacious and safe treatment for steroid-refractory cGVHD. Longer follow-up is necessary in this study in order to determine the durability of response and survival.

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DECODING OF THE GENOME WIDE microRNA-mRNA INTERACTION MAPS WITH HITS-CLIP REVEALS A DISTINCT miRNAOME IN allo-ANTIGEN ACTIVATED T CELLS

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Following allogeneic bone marrow transplantation (BMT), donor T cells that respond to allo-antigens cause GVHD and those that respond to non-alloantigens are critical for immune-reconstitution. A genome wide molecular landscape of the T cells responding to alloantigens and the non-alloantigens and whether they are distinct is not known.

MicroRNAs (miRs) are critical molecular regulators; miR activity requires base pairing with messenger RNA (mRNA). But predicting target mRNAs is a challenge. High-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) identifies functional protein-RNA interaction sites. We mapped genome-wide protein-RNA binding sites to covalently crosslink native argonaute (Ago) protein-RNA complexes in alloantigen activated and non-alloatigen, CD3+antibody activated T cells. Ago HITS-CLIP pulls down AGO proteins along with miRs and associated mRNAs with subsequent application to miR and mRNA arrays (RIP-Chips) identify clinically relevant miR-mRNA interactions.

Forty five miRs were differentially enriched in activated T cells, either allo-T cells or CD3-ab-stimulated T cells that were divided into 4 groups. Amongst them, only 19 miRs were enriched in allo-T cells when compared to CD3 antibody-treated T cells. Six of the 19 (miR-142-3p, 142-5p, 16, 29a, 66d and 669i) were selected for further analysis, and their transcript enrichments were screened by Affymetrix microarrays for mRNAs. A total of only 1226 genes were expressed while 200 genes were differentially expressed in allo-T cells and 288 genes in CD3-ab-T cells. We next cross-linked the data from RIP-Chip-miRs and mRNA affymetrix to target prediction programs and hypothesized that reduced miR enrichments should be companied with decreased mRNA enrichments. We found that Fchsd2, Wapal and MLL3 genes were predicted to be targets of miR-16, Cyb5r3 as miR-142-3p target, Rcor1 as miR-29a, Synji1 and Med17 as miR-142-5p, LPP as miR-669d, Dock9 as miR-660i and Rcor1 as target of miR-669i respectively.

Validation studies with RT-qPCR and western blot confirmed the patterns of enrichment of the miRs -16, 142-3p, 142-5p and 29a and the predicted targets Wapal and Fchsd2. This pattern was also confirmed in vivo following BALB/c into B6 BMT.

In summary, we demonstrate for the first time distinct miR-mRNA interaction map based on genome wide analysis with the state of art HITS-CLIP and identified specific targets in allo-activated T cells.

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TCR TRANSDUCTION APPROACH TO EXPAND SEVERE GRAFT-VERSUS-HOST DISEASE INDUCING CD4 SPECIFIC T CELLS

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Allogeneic hematopoietic cell transplantation (HCT) remains the most promising curative therapy for the treatment of hematological malignancies, but is complicated by the development of graft-versus-host disease (GVHD), mediated by donor T cells targeted against either HLA or minor histocompatibility antigens (miHA) of the host. CD4 T cells recognizing certain miHA have been found capable of mediating severe GVHD on their own in some murine models. Previous studies have utilized CDR3-size spectratyping to identify a unique population of CD4+Vβ11+ T cells that have subsequently been shown capable of infiltrating both the rete-like prominences of the dorsal lingual epithelium and the ileal crypts of the small intestine while mediating lethal GVHD in the miHA disparate C57BL/6 (B6) → C.B10-H2b/LiMcdJ (BALB.B) transplantation model. The unique and restricted BALB.B specific T cell response of the B6 CD4+ Vβ11+ T cells indicates a limited number of miHA are likely responsible for the lethal GVHD observed. Sequencing of the skewed bands from CD4+ Vβ11+spectratype analysis of BALB.B recipients with GVHD indicated a strong prevalence (65%) of the T cell receptor (TCR) Jβ 2.5, implying a dominant alloreactive expansion within this specific CD4+ Vβ11+ T cell family. However, a better understanding of the pathogenesis of this particular response has been hampered by the insufficient quantity of T cells expressing the specific miHA-reactive TCR. To facilitate further study of this CD4 response, we have utilized TCR transduction of bone marrow from B6.129P2-Tcrbtm1Mom/J TCR β-chain knockout mice (TCR β -/-) mice with GFP-tagged DNA sequence, which we then transplanted back into lethally irradiated TCRβ -/- mice to generate an efficient source of large numbers of this minute population of reactive, GVHD-associated Vβ11+ T cells. We conclude that TCR transduction can be utilized to generate large numbers of highly specific, reactive T cell populations for use during in vitro or in vivo experiments to characterize previously inaccessible populations of GVHD mediating T cells.