Background: Metastatic solid tumors, especially neuroblastoma (NB) and Ewing's sarcoma (ES) have dismal prognosis (Perkins et al, *PLoS One*, 2014). Targeted cellular therapy with T or NK cells modified with chimeric antigen receptors (CAR) is a novel approach to chemo-resistant childhood solid tumors (Grupp SA, *Clin Cancer Res*, 2012; Mackall C, *Front Oncol*, 2012). NK cells can be significantly expanded by co-culture with genetically engineered K562 cells overexpressing mb-IL21 (Lee D, PLOS, 2012). Riddell and colleagues have identified ROR1 as a novel target on B cell tumors in which CARs can be developed and utilized for targeted cellular therapy (Riddell S, *Blood*, 2010).
Objective: To evaluate the in-vitro NK cytotoxic activity and NK cell function following ex-vivo K562 mb-IL21expansion of PBNK nucleofected with anti-ROR1-CAR against NB and ES.
Material and Methods: PBNK were expanded with lethally irradiated K562 Clone 9.mb-IL21 (generously provided by Lee D, MD, PhD, MD Anderson, TX). Ex-vivo expanded PBNK (ExPBNK) cells were electroporated with anti-ROR1-CD28-41BBl-CD3z-tEGFR—mRNA (Anti-ROR1-CAR was generously provided by Riddell S, MD, Fred Hutch, WA). Anti-ROR1-CAR-NK expression was detected using anti-mouse IgG, $F(ab')_2$. Anti-ROR1-CAR-NK cytotoxicity was investigated against NB (SKNBE2, SKNFI & SHSY5Y) and ES (TC71, EWS 502 & A673) cell lines by DELFIA cytotoxicity assay at 10:1 E: T ratio. Intracellular staining of CD107a, Interferon Gamma, Perforin and GranzymeB was performed using a 10:1 E:T ratio of anti-ROR1-CAR-NK cells against tumor targets & analyzed on the MACSQuant flow cytometer.
Results: NB and ES cell lines expressed ROR1 (50.2±15.6%) and (31.5±12%), respectively. Expansion of NK cells was significantly increased 3988±435 fold (p=0.00001) at day 14 vs day 0. Nucleofection success was measured by $F(ab')_2$ expression and showed a significant increase in anti-ROR1-CAR- (88.3±1.69%) vs Mock-electroporated NK cell populations (8.1±6.9%) p= 0.0001 at 36-48 hours (Figure 1). ROR1 negative A673 served as negative control. Anti-ROR1-CAR-NK significantly increased cell lysis compared to Mock NK (93.1±1.9% vs 62.8±5.2%, p=0.0008) against ROR1expressing cell lines at 10:1 ET ratio (Figure 2). Similarly, expression of CD107a (46.9±2.2 vs 26.9±3%) p=0.0004, Interferon Gamma (36.9±12.4vs15.3±6.9%) p=0.008, GranzymeB (62.8±4.4 vs 46.6±7.7%) p=0.003, and Perforin (50.5±8 vs 32.7±14.1%) p=0.004 were significantly increased in anti-ROR1-CAR-NK vs Mock-NK cells at 10:1 E:T ratio against the ROR1 expressing targets.
Conclusion: Anti-ROR1-CAR-ex-PBNK cells had significant enhanced cytotoxicity and significantly increased CD107a, Interferon Gamma, Perforin, and GranzymeB activity against ROR1 expressing pediatric solid tumors. Future directions include investigating the ex-PBNK anti–ROR1-CAR cells in-vivo against ROR1 expressing pediatric solid tumors.

95

Hematopoietic Stem Cell Transplantation in Primary Immunodeficiencies in Brazil-a Survey of the Working Group on Paediatric Transplantation of the Brazilian Society of Bone Marrow Transplantation

Juliana Folloni Fernandes [1,2], *Adriana Seber* [3], *Liane Esteves Daudt* [4], *Lisandro Ribeiro* [5], *Victor Zecchin* [6], *Marcos A. Mauad* [7], *Luiz Guilherme Darrigo Jr.* [8], *Ana Karine Vieira* [9], *Lauro Gregianin* [10], *Adriana Martins Sousa* [11], *Adriana Santini* [4], *Rita Tavares* [12], *Samantha Nichele* [5], *Adriana Koliski* [13], *Luis Fernando S. Bouzas* [14], *Nelson Hamerschlak* [15], *Carmem Bonfim* [5]. [1] *Instituto da Criança-HCFMUSP, Sao Paulo, Brazil;* [2] *Hospital Israelita Albert Einstein, Sao Paulo, Brazil;* [3] *Instituto de Oncologia Pediatrica, Sao Paulo, Brazil;* [4] *Hospital das Clínicas de Porto Alegre, Porto Alegre, Brazil;* [5] *Bone Marrow Transplantation Unit, Federal University of Paraná, Curitiba, Brazil;* [6] *Instituto de Oncologia Pediátrica, Sao Paulo, Brazil;* [7] *Bone Marrow Transplantation, Centro de Oncologia e Hematologia, Jau, Brazil;* [8] *Hospital das Clínicas—USP—Ribeirão Preto, Ribeirão Preto, Brazil;* [9] *Universidade Federal de Minas Gerais, Belo Horizonte, Brazil;* [10] *Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil;* [11] *Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil;* [12] *Instituto Nacional de Cancer, Centro Nacional de Transplate de Medula Ossea-CEMO, Rio de Janeiro, Brazil;* [13] *Paediatric Intensive Care Unit, Federal University of Parana, Curitiba, Brazil;* [14] *National Institute of Cancer INCA, Rio de Janeiro, Brazil;* [15] *Hematology and Bone Marrow Transplantation Dept, Hospital Israelita Albert Einstein, Sao Paulo, Brazil*

Hematopoietic Stem Cell Transplantation (HSCT) is curative in a large number of primary immunodeficiencies (PID). The first transplant in PID in Brazil was held in 1992 at the Hospital de Clínicas da Universidade Federal do Paraná. Since then, in Brazil, only few centers have performed transplants in these pathologies. Objective: Retrospective analysis of outcomes of HSCT performed in patients with PID in 10 Brazilian centers participating in the working group of pediatric transplantation of the Brazilian Society of BMT (SBTMO). From 1992 to April 2014, 166 patients underwent HSCT on PID in 10 different centers in Brazil, mostly in the Hospital of UFPR (n = 90), followed by the Hospital Israelita Albert Einstein (n = 27) and the other centers with 10 or fewer. Most patients were male (M = 130, F = 36), and most patients had age lower than 3 years (n = 119). The diagnoses were distributed among: Wiskott-Aldrich syndrome (n = 56); Severe Combined Immunodeficiency (n = 52); Chronic granulomatous disease (n = 14); Familiar Hemophagocytic lymphohistiocytosis (n = 13); Chediak-Higashi Syndrome (n = 12); Hyper-IgM syndrome (n = 5); Severe congenital neutropenia (n = 5); IPEX syndrome (n = 3); combined immunodeficiency disease (n = 2); Leukocyte Adhesion Deficiency (n = 2); Deficiency of IFN-gamma (n = 1) and Griscelli syndrome (n = 1). Thirty-two patients received transplants from identical sibling donors, 112 unrelated donors, and 22 other family donors (10/10 compatible or haploidentical). Graft was bone marrow in 84 cases, umbilical cord blood in 78 cases and 4 patients received stem cells from peripheral blood. Ninety-nine patients received myeloablative transplants (including busulfan>8mg/Kg), 55 patients received reduced toxicity conditioning and 12 patients received no conditioning. The prophylaxis of graft-versus-host disease was performed with cyclosporine A in most cases, associated with mycophenolate mofetil, methotrexate or steroids. Overall survival was 68.7% at 3 years. In univariate analysis, there was no statistical difference between gender, diagnosis, age at HSCT, year of transplant, source of cells, or donor type. Among the most frequent pathologies groups, analysis was done in 2 groups, the severe combined immunodeficiency (n = 52) and the Wiskott-Aldrich syndrome (n = 56) Syndrome. In these subgroups, the overall survival at 3 years was 60% and 79%, respectively.
Conclusion: This is the first survey made on transplants on primary immunodeficiencies in Brazil. Despite the difficulties in diagnosing and referral of these children for special treatment in our country, we have seen that the overall survival of our patients is not different than those reported in international studies in Europe or North America. This survey is extremely important to have the results of these cases in Brazil, and identify areas to improve the outcomes of transplants in lower income countries.

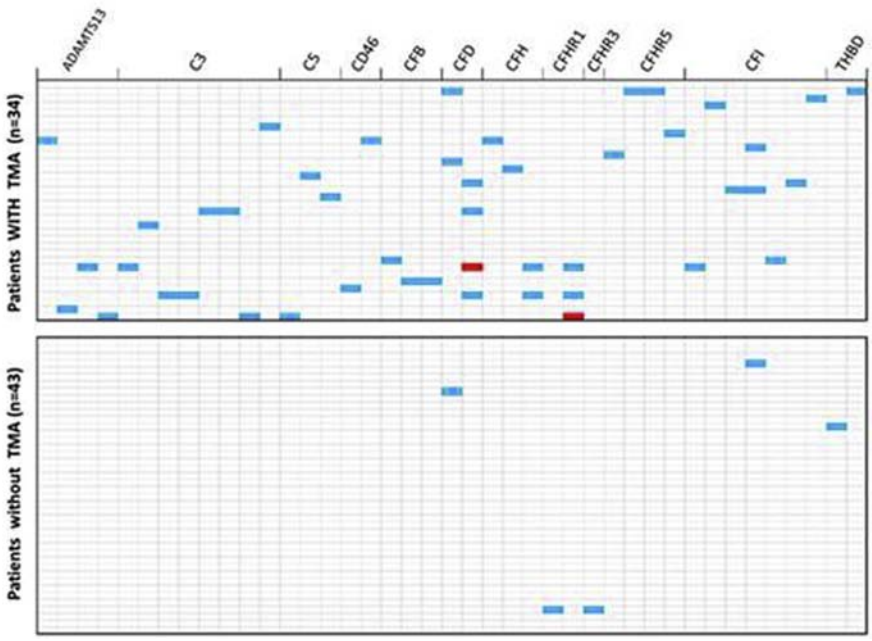

Figure. Genetic variations in complement associated genes (HSCT recipient DNA). Figure shows a "heat map" of gene variants in recipients with and without TMA: each column represents a gene, with heterozygous variants shaded light blue and homozygous variants shaded red. Each row is a single patient, illustrating the wide distribution of variants in multiple genes in the TMA cases, while few variants are seen in the cases without TMA.

96

The Genetic Fingerprint of Susceptibility to Transplant Associated Thrombotic Microangiopathy

Sonata Jodele [1], *Benjamin Laskin* [2], *Kejian Zhang* [3], *Fanggeng Zou* [3], *Christopher E. Dandoy* [1], *Kasiani C. Myers* [1], *Javier El-Bietar* [1], *Gregory Wallace* [1], *Michael S. Grimley* [1], *Jack Bleesing* [1], *Jens Goebel* [4], *Diane Kissell* [5], *Shannon Nortman* [5], *Adam Lane* [1], *Stella M. Davies* [1]. [1] *Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;* [2] *Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;* [3] *Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;* [4] *Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center, Cincinnati, OH;* [5] *Molecular Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Background: Transplant associated thrombotic microangiopathy (TMA) is frequent after HSCT, and in severe cases causes significant morbidity and mortality. Currently there are no data addressing individual susceptibility to transplant associated TMA. Frequent genetic variants in ADAMTS13 and complement genes have been described in other microangiopathies such as aHUS and TTP. We hypothesized that polymorphic variants in complement genes increase susceptibility to transplant associated TMA.

Methods: 90 consecutive allogeneic HSCT patients were enrolled on a prospective TMA biomarker study and categorized as having TMA or no TMA using rigorous diagnostic criteria (Jodele et al, Blood 2014). Genomic DNA was isolated from pre-HSCT recipient blood. We used a candidate gene approach to identify 12 genes within the complement pathway likely to play a role in terminal complement activation, the likely effector mechanism for vascular damage in TMA. All exons, flanking intronic and untranslated regions of ADAMTS13, CFH, CFI, CFB, MCB, THBD, C3, C5, CFD, CFHR1, CFHR3 and CFHR5 were sequenced using next generation sequencing technology. The resulting sequence reads were aligned against the reference DNA sequence and the variants were detected using NextGENe software. Observed variants were compared against dbSNP (NCBI). Novel variants were further evaluated using laboratory developed bioinformatic tools. Eight variants previously described as likely pathogenic and 34 variants of unknown pathogenic significance were identified. Results were compared in patients with and without TMA.

Results: 77 patients had DNA available for analysis (34 with TMA and 43 without TMA); 23 of 34 (68%) subjects with TMA had complement gene variants as compared to 4 of 43 (9%) controls without TMA ($p<0.0001$). Figure shows a "heat map" of gene variants in recipients with and without TMA illustrating the wide distribution of variants in multiple genes in the TMA cases, while few variants are seen in those without TMA. Likely pathogenic variants previously described in other microangiopathies were seen in ADAMTS13 (n=2), CFH (n=2), CFB (n=1), CFHR5 (n=1) and CFI (n=3), all in recipients with TMA. No known pathogenic variants were seen in subjects without TMA. Two recipients with TMA had homozygous deletion of CFHR3/R1, which was not seen in the recipients without TMA. The median number of gene variants (of known or unknown significance) seen in recipients with TMA was 1 (0-7), and 0 (0-2) in those without TMA ($p<0.0001$). Two subjects had 5 variants and one 7 variants and all three had fatal TMA.

Conclusion: The incidence of complement gene variants was higher in patients with TMA as compared to patients without TMA, indicating that genetic susceptibility importantly alters risk. Additional larger studies are needed to define the mechanistic importance of variants of unknown clinical significance.

97

High Hematopoietic Transplantation Comorbidity Index Is Not Associated with Increased Transplant Related Mortality: Review of a Large Cohort of Pediatric Patients Undergoing Allogeneic Stem Cell Transplantation Following Busulfan-Based Regimens for Malignant and Non-Malignant Diseases

Justine Kahn [1], *Naima Al Mulla* [2], *Mahvish Qureshi* [1], *Grace Kim* [1], *Zhezhen Jin* [3], *Monica Bhatia* [4], *Esra Karamehmet* [5],