Conclusions: An association of 3 membrane proteins expressed on EVs and onset of aGVHD was observed. Of note, CD146, CD44 and CD31 belong to the Cell Adhesion Molecule Family and are crucial for endothelium and immune cells interactions. MicroRNAs play critical roles in several biological processes affecting T-cells. Hence, miRNAs are been investigated as potential biomarkers for GvHD. The functional role of miR-194 in GVHD pathogenesis remains to be determined (Gimondi S Exp Hematol, 2016) while miR-100 has been reported to limit neovascularization in the intestine during GvHD (Leonhardt F Blood 2013).

CO037

THYMIC FUNCTION AND T CELL HOMEOSTASIS AFTER HAPLOIDENTICAL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

S. Butera[1], G. Lia[1,3], A. Evangelista[4], L. Tosti[1,3], L. Comba[1,3], S.Cena[1,3], R. Pulito[1] L. Brunello[1,3], M. Gilestro[1], M. Ruggeri[1], D. Oddolo[1], S.A. Caltagirone[1], V.E. Muccio[1], E. Saraci[1], M. Festuccia[1,2], G. Ciccone[4], E. Maffini[1,3], C. Dellacasa[1], M. Boccadoro[1,2], L. Giaccone[1,2], A. Busca[1], P.M. Omedè[1], B. Bruno[1,3]

[1]A.O.U. Città della Salute e della Scienza di Torino, Dipartimento di Oncologia e Ematologia, SSD Trapianto Allogenico di Cellule Staminali; [2]A.O.U. Città della Salute e della Scienza di Torino, Dipartimento di Oncologia e Ematologia; [3]Università degli Studi di Torino, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute; [4]A.O.U. Città della Salute e della Scienza di Torino, Clinical Epidemiology, Italy

Introduction: Post-transplant cyclophosphamide (PTCY) has expanded the application of haploidentical stem cell transplantation (haplo-HSCT). Thymic function may play a pivotal role in long-term clinical outcomes.

Methods: ETwenty-nine patients (median age 53, r 28-70) with hematological malignancies underwent haplo-HSCT with PTCY. Blood samples were collected before conditioning and at 1, 3, 6, 12, 18, 24 months after transplant. Analyses of CD4+ and CD8+ T-cell subsets by flow-cytometry were correlated with Real-Time PCR quantification of signal joint T-cell receptor excision DNA circles (sjTRECs), specific marker of naïve T-cells thymopoiesis. A) Naïve; b) central; c) memory; and d) revertant T cells were defined as follows: a) CD4+CD45RA+ CD62L+; b) CD4+CD45RO+CD62L+; c) CD4+CD45RO+CD27-; and d) CD4+CD45RA+/45RO+, respectively. SjTRECs real-time PCR was performed on genomic DNA extracted from sorted CD4 and CD8 T-cells. Associations between sjTRECs and T-cell subsets and trends over time were evaluated by Generalized Linear Models.

Results: Following PTCY induced T-cell depletion, a constant gradual increase in absolute numbers of all T-cell subsets and of sjTRECs from the first month up to two years post-transplant was observed. Table 1 summarizes: a) patient median counts of CD4+ and CD8+ T cell subsets at 1, 3, 6, 12, 15, 24 months; b) median values of sjTREC copies/100 ng DNA from sorted CD4+ and CD8+ T cells; c) median values of healthy donor T cell counterparts and sjTREC copies respectively. Overall, at two years, CD4 and CD8 T-cell levels and sjTRECs levels were lower than those observed in healthy donors. Molecular analysis of the sjTRECs kinetics was associated with the increase in CD4+ naïve T-cells (global p<0,008). This correlation clearly suggests that most of naïve T-cells derives from thymic re-education of donor precursor stem cells. Furthermore, an increase in revertant memory T-cells was also significantly correlated with sjTRECs kinetic (p 0,041 and <0.001 respectively). By contrast, central and effector memory T-cells showed a faster thymic-independent expansion. Importantly, sjTRECs levels and thymic dependent immune-reconstitution were higher in a cohort of 63 adult patients undergoing HSCT from HLA identical donors (data not shown). The impact of clinical parameters was evaluated on thymic function from 6 months after transplant onwards. By multivariate analysis, low baseline TRECs values, moderate-severe chronic GVHD, age older than 50 years old were significantly associated with low thymic output after haplo-HSCT.

Conclusions: Active thymic function despite age-dependent involution substantially contributes to T-cell reconstitution after haplo-HSCT. Lower production of sjTRECs as compared after HLA identical sibling transplants may partly be due to a higher degree of "mismatching" of MHC molecules during thymic re-education. Chronic GVHD and older age are significantly correlated with thymic activity.

Table 1.

	Time after HSCT (months)							
CD4⁺ subset (count/μl)	**preTx**	**1**	**3**	**6**	**12**	**18**	**24**	**donors**
Naïve CD45RA⁺CD62L⁺	41,7	1,9	2,3	5,5	9,3	31,9	40,2	199,6
Revertant memory CD45RA⁺45RO⁺ CD62L⁺	53,4	2,7	7,0	13,0	18,3	24,5	40,4	131,1
Central Memory CD45RO⁺CD62L⁺	82,9	17,6	50,7	89,8	127,0	202,8	197,6	161,9
Effector memory CD45RO⁺CD62L⁻	37,6	6,9	32,3	61,2	186,6	122,0	102,0	91,6
CD8⁺ subset (count/μl)								
Naïve CD45RA⁺CD62L⁺	24,8	2,2	10,1	50,4	75,5	83,7	63,7	115,1
Revertant memory CD45RA⁺45RO⁺ CD62L⁺	49,5	16,1	29,1	63,7	88,2	128,3	112,8	34,7
Central Memory CD45RO⁺CD62L⁺	25,4	29,5	13,5	36,0	70,3	96,7	52,5	17,8
Effector memory CD45RO⁺CD62L⁻	56,9	2,5	11,8	86,6	148,0	277,3	253,3	40,6
sjTRECs (copy/100ng gDNA)								
CD4⁺	7,5	-	2,8	5,9	36,2	25,8	22,4	84,6
CD8⁺	5,3	-	1,6	4,6	8,9	13,6	8,2	77,9

CO038

MULTIFACETED IMMUNE CHECKPOINT EXPRESSION AND SENESCENT MARKERS IMPAIRS BONE MARROW Vγ9Vδ2 T- CELL FUNCTION IN MULTIPLE MYELOMA PATIENTS

B. Castella, C. Riganti, M. Foglietta, E. Tripoli, C. Giannotta, M. Massaia

Department of Molecular Biotecnology and Health Sciences, University of Torino; Center for Experimental Research and Medical Studies (CeRMS), A.O.U. Città della Salute e della Scienza di Torino; SC Ematologia, AO S.Croce e Carle di Cuneo, Italy

Introduction: Malignant B cells are privileged targets of Vγ9Vδ2 T-cell recognition and killing *in vitro*, but this predisposition is lost *in vivo* due to multiple mechanisms mediated by tumor cells and the tumor microenvironment (TME). By interrogating the phosphoantigen (pAg) reactivity of bone marrow (BM) Vγ9Vδ2 T cells from multiple myeloma (MM) patients, we have unravelled a very early and long-lasting Vγ9Vδ2 T-cell immune dysfunction sustained by several mechanisms, including a multifaceted immune checkpoint (ICP) expression in the TME. Notably, single-agent PD-1 blockade is insufficient to recover anti-myeloma immune responses mediated by BM Vγ9Vδ2 T cells, indicating the existence of primary and adaptive resistance to ICP blockade.

Methods: The expression of ICP and senescent markers by BM Vγ9Vδ2 T cells in multiple myeloma patients was examined by multicolor flow cytometry. Vγ9Vδ2 T-cell functions was evaluated *in vitro* using the appropriate stimulation and combinations of different anti-ICP mAbs. Western blot analysis has been used to analyze the signal transduction pathways triggered by PD-1 (PI3K/Akt and Ras/MEK/ERK) and TIM-3 (JAK/STAT) in BM Vγ9Vδ2 T-cell after pAgs stimulation.

Results: We have observed a significant up-regulation of alternative inhibitory receptors (TIM-3 and TIGIT) in MM BM Vγ9Vδ2 T cells after pAg stimulation and PD-1 blockade. The combination of PD-1 and TIM-3 blockade improves the recovery of BM Vγ9Vδ2 T-cell proliferation. To investigate the molecular mechanisms implied in the alternative ICP up-regulation driven by single PD-1 blockade, we have analyzed the signal transduction pathways triggered by PD-1 (PI3K/Akt and Ras/MEK/ERK) and TIM-3 (JAK/STAT) in BM Vγ9Vδ2 T cells under resting conditions and after pAg stimulation. We have identified a remarkable down-modulation of pAKT, pJAK1 and pSTAT1, which may contribute to the exacerbation of Vγ9Vδ2 T-cell dysfuntion. We have also documented a significantly down-modulation of the transcription factor T-bet as an additional hallmark of BM Vγ9Vδ2 T-cell exhaustion. These senescent tumor-experienced Vγ9Vδ2 T cells with enhanced resistance to PD-1 blockade are phenotypically characterized by CD160 expression and lack of CD28 expression.

Conclusions: Deciphering the immune suppressive mechanisms involved in BM Vγ9Vδ2 T-cell anergy of MM patients can be very infor-

mative to develop effective interventions to fully exploit the immune potency of Vγ9Vδ2 T cells in B-cell malignancies.

CO039

GENOME WIDE METHYLATION IN HEMATOPOIETIC STEM CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

S. Trino[1], L. De Luca[1], A. Caivano[1], I. Laurenzana [1], P. Zoppoli[1], F. La Rocca[2], G. Calice[1], V. Simeon[3], D. Memoli[4], A. Weisz[4], D. Cilloni[5], G. Falco[6], A.M. Carella[7], L. Del Vecchio[8,9], P. Musto[10]

[1]Laboratory of Preclinical and Translational Research, IRCCS – Referral Cancer Center of Basilicata (CROB), Rionero in Vulture (PZ); [2]Laboratory of Clinical Research and Advanced Diagnostics, IRCCS – Referral Cancer Center of Basilicata (CROB), Rionero in Vulture (PZ); [3] Medical Statistics Unit, University of Campania "Luigi Vanvitelli", Naples; [4]Laboratory of Molecular Medicine and Genomics, Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi (SA); [5]Department of Clinical and Biological Sciences, University of Turin, Orbassano (TO); [6]Department of Biology, University of Naples Federico II, Naples; [7]Hematology and Bone Marrow Transplant Unit, IRCCS - Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG); [8]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Napoli; [9]CEINGE Biotecnologie Avanzate scarl, Naples; [10]Scientific Direction, IRCCS – Referral Cancer Center of Basilicata (CROB), Rionero in Vulture, Italy

Introduction: Allogeneic hematopoietic stem cell transplantation (AHSCT) is a curative therapeutic approach for different hematological diseases. Little is known, however, about the biology of HSCs after transplant in terms of homing, self renewal and differentiation. DNA methylation is involved in HSC stemness and commitment, thus potentially exerting a role in the reconstitution of hematopoietic system after AHSCT. We explored DNA methylation dynamics in bone marrow (BM) HSCs after transplant.

Methods: We enrolled 10 patients, respectively affected by AML (n=6), ALL (n=3), and HL (n=1), and receiving BM AHSCT. CD34+ cells were sequentially collected from BM of donors (t0) and matched recipients [day 30, 60, 120, 180, 360 (t1-t5)]; DNA was used for array-based methylation (Infinium HumanMethylationEPIC, Illumina). A schematic overview of the study design is reported in Figure 1.

Results: Genome wide methylation profiles of donors and recipients, interrogating 850.000 CpG sites, were generated. We firstly performed an unsupervised hierarchical clustering observing that, generally, each patient profiled into a specific methylation cluster; some donors clustered together, while others separated with their respective recipient. Of note, global methylation level was similar in all times (t0-t5), when considering all samples. Subsequently, to assess methylation changes after transplant, we compared methylation profiles of donor and recipient HSCs. First, we identified 12043 differentially methylated probes (DMPs) in t1 vs t0, mapping to 1857 genes (1380 hyper- and 477 hypo-methylated). Interestingly, hypo-methylated genes were involved in immune responses, resistance to host and allograft rejection, while hyper-methylated genes were enriched in cell remodeling, adhesion and cell activation. As expected, both hypo- and hyper-methylated genes enriched in function related to hematopoiesis, such as leukocyte activation and differentiation. Thus, 30 days after AHSCT, CD34+ cells consistently modified their methylation pattern. When we evaluated DMPs at other time points respect to donors, their number decreased indicating that methylation levels tend to normalize during transplant. To verify this, we evaluated: (i) differentially methylated genes both in t1 and t5 against t0, representing a long term modification, and (ii) differentially methylated genes at t1, but not in t5, against t0, having a short term effect only present in t1. We identified only 270 differentially methylated genes, while the majority (n=1699) showed a methylation profile similar to donors.

Conclusions: Our study shows that HSC methylation pattern consistently changes in an early time after AHSCT, possibly as a consequence of the adaptation of donor cells in recipient BM niche. Moreover, after a year, HSCs prevalently restore methylation patterns similar to donor cells at baseline. Ongoing studies on status and dynamic of HSC methylation could define its role in AHSCT outcome.

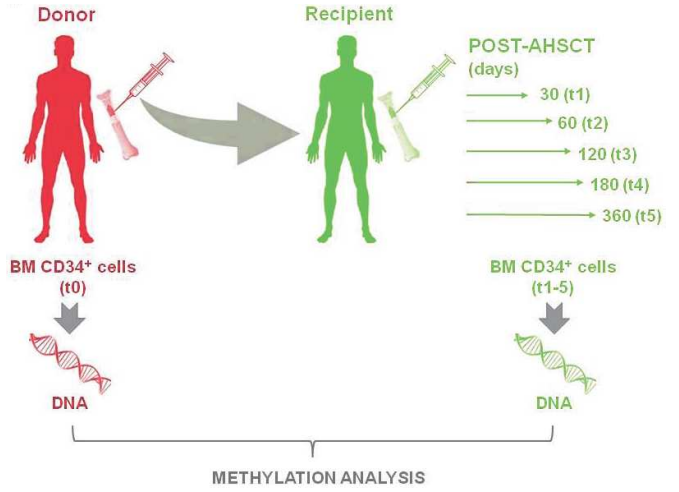

Figure 1.

CO040

ABCA1: AN UNEXPECTED ROLE IN Vγ9Vδ2 T-CELL ACTIVATION BY DENDRITIC CELLS

M. Massaia, C. Riganti, M. Foglietta, E. Tripoli, B. Castella

Department of Molecular Biotecnology and Health Sciences, University of Torino; Center for Experimental Research and Medical Studies (CeRMS), A.O.U. Città della Salute e della Scienza di Torino;SC Ematologia, AO S.Croce e Carle di Cuneo, Italy

A very peculiar feature of Vγ9Vδ2 T cells is their ability to recognize pyrophosphorylated isoprenoids [i.e, phosphoantigens (pAgs)], generated in the mevalonate pathway of mammalian cells. Isopentenyl pyrophosphate (IPP) is the prototypic pAg recognized by Vγ9Vδ2 T cells. Tumor cells and dendritic cells (DCs) are privileged targets of Vγ9Vδ2 T cells because they produce significant amounts of IPP which can be boosted with zoledronic acid (ZA), the most potent aminobisphosphonate clinically available and a strong inhibitor of farnesyl pyrophosphate synthase in the Mev pathway. ZA-treated DCs generate and release in their supernatants picomolar IPP concentrations which are sufficient to induce the activation of Vγ9Vδ2 T cells. We have recently shown that the ATP-binding cassette transporter A1 (ABCA1) plays a major role in the extracellular release of IPP from ZA-treated DCs. This novel ABCA1 function is fine-tuned by physical interactions with IPP, apolipoprotein A-I (ApoA-I) and butyrophilin-3A1 (BTN3A1). IPP binds to ABCA1, BTN3A1, and apoA-I, further promoting interactions between these molecules. We speculate that the aim of this mènage à trois is two-fold: the first is to extend the range of immune regulation to Vγ9Vδ2 T cells that are not in close proximity to pAg-presenting cells; the second to protect pAg-presenting cells from apoptosis due to intracellular accumulation of the pro-apoptotic ATP analog ApppI. A BCA1 can work as a safety valve instructed to avoid that Vγ9Vδ2 T-cell activation is prematurely terminated by the apoptotic death of pAg-presenting cells. These data further strengthen the close relationship between lipid metabolism and immune function.