Acute Skin GVHD

Previous studies have suggested the immunomodulatory effects of vitamin D may play a role in ameliorating acute GVHD pathogenesis. Vitamin D has been shown to suppress T_h1 and T_h17 cytokine production, inhibit differentiation and maturation of dendritic cells, promote T_h2-cell development, and increase expression of regulatory T-cells. We performed a retrospective analysis to evaluate whether serum vitamin D levels on day 30 after HSCT are predictive of organ-specific GVHD.

Methods: Fifty-four patients undergoing allogeneic HSCT at the University of Pennsylvania between January 2008 and December 2012 were included in the analysis. Serum 25-hydroxyvitamin D (25OHD) concentrations were determined on day 30 following HSCT. We analyzed patients in two groups according to their median day-30 25(OH)D concentration (<20 and ≥20 ng/mL). The associations between vitamin D levels and other variables were conducted using Pearson correlations and t-tests. We then used a landmark analysis to estimate the impact of day 30 vitamin D levels on subsequent clinical outcomes. Univariate analyses were performed using cumulative incidence and Cox regression analyses. Multivariable models were constructed using the backward elimination method. We also conducted immunophenotyping of day-30 peripheral blood samples on patients who had low vs. normal vitamin D levels.

Results: The median 25(OH)D concentration on day 30 was 20 ng/mL (range 6 - 50), reflecting severe vitamin D deficiency in half of the patients. Vitamin D levels significantly correlated with age, disease type, need for TPN during transplant and day-30 albumin levels. In multivariate analysis of outcomes, day-30 vitamin D levels inversely correlated with risk of acute skin GVHD (HR 0.27; 0.07 − 1.01; p=0.05). This association was specific to patients undergoing reduced-intensity conditioned (RIC) HSCT (p<0.001) and not myeloablative HSCT (p=0.44). Vitamin D deficient patients expressed > 4-fold higher levels of CCR4, a skin-homing receptor, on peripheral blood T-cells as detected on flow cytometric analysis (p=0.036). Day 30 vitamin D levels did

not significantly impact the risk of gastrointestinal (HR 2.16; 0.76 − 6.09; p=0.15) or hepatic GVHD (HR 0.31; 0.078 − 1.24; p=0.1). No differences were observed in overall acute grade 2-4 GVHD, chronic GVHD, OS or non-relapse mortality.

Conclusion: Vitamin D deficiency on day 30 after allogeneic HSCT is associated with significantly increased risk of grade 2-4 cutaneous acute GVHD and increased expression of CCR4 on peripheral blood T-cells in patients undergoing RIC HSCT. Vitamin D may confer a protective effect against acute skin GVHD via reduction in CCR4 expression.

417

Differences Between Gvhd and GVL May be Rather Quantitative and Not Dependent on a Malignant Transformation of the Target Cell

Martin Gramatzki[1], Andreas Guenther[1], Natalie Schub[1], Michael Kneba[2], Andreas Humpe[1]. [1] Division of Stem Cell Transplantation and Immunotherapy, Kiel, Germany; [2] 2nd Department of Medcine, University of Kiel, Kiel, Germany

The therapeutic potential of allogeneic stem cell transplantation (allo-SCT) in patients with malignant diseases of the hemato-lymphatic system is thought to depend apart from conditioning with high dose therapy on the anti-tumor effect provided by the immune system of the donor. However, the immune system of the donor may exert severe and difficult to control graft-versus-host-disease (GvHD). Efforts to dissect GvHD and graft versus-leukemia/lymphoma reactions (GvL) have remained dissatisfying. Here, we report immune reactions observed after allo-SCT suggesting that GvHD and GvL are related rather quantitatively and correspond with achieving 100% donor chimerism allowing the extinction of remaining host immune cells regardless whether malignant or not. Clinical findings: A patient wit relapsed T-PLL was treated with allo-SCT and received stem cells from a matched unrelated donor (MUD). After being in complete cytological and molecular remission she relapsed molecularly on day +351. The molecular analysis revealed malignant cells at the level of 0.16% in the blood. While alemtuzumab was used for bridging, on day +383 a first donor lymphocyte infusion (DLI) was applied. Despite a second and third DLI application later, a steady increase in the MRD level above 2x10-2 was noted. However, the fourth DLI application on could not be given because the patient had developed acute GvHD of the skin. At the same time MRD no longer was detectable by RQ-PCR with a detection limit below 1x10-5. GvHD resolved upon treatment and the patient is in continuous complete remission for more than 5 years. Likewise, a quantitative effect in the balance between donor and host immune cells was observed when a patient received an ABO blood group major incompatible graft from a MUD. Frequently, in those situations a delayed recovery of the major incompatible erythropoiesis is observed due to persisting isohemagglutinin producing donor cells. Here, even after complete tapering of the immunosuppression and therapeutic approaches including rituximab anemia persisted. After DLI application, the patient developed acute GvHD of the skin but consecutively the anemia resolved. Conclusion: In these informative situations DLI documented their efficacy in correcting a misbalanced immune system after allo-SCT. While in the MRD situation remaining tumor cells vanished with increasing doses of DLI exactly when first signs of GvHD occurred, in the ABO incompatible scenario few remaining host immune cells producing isohemagglutinins were eradicated with a small dose of DLI. This leads to the conclusion that donor immunosurveillance

may be independent of whether the phenotype of the donor immune cells is malignant and that GvHD and GvL may be more a quantitative than qualitative effect.

418

Ocular Gvhd: Epidemiology, Risk Factors and Impact on Quality of Life-a Chronic Gvhd Consortium Study

Madan H. Jagasia[1], Xiaoyu Chai[2], Joseph Pidala[3], Yoshihiro Inamoto[4], Mukta Arora[5], Corey S. Cutler[6], Mary E.D. Flowers[7], Laura Johnston[8], Steven Z. Pavletic[9], Stephanie J. Lee[4]. [1] Vanderbilt University Medical Center, Nashville, TN; [2] Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA; [3] H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; [4] Fred Hutchinson Cancer Research Center, Seattle, WA; [5] Hematology, Oncology and Transplant, University of Minnesota, Minneapolis, MN; [6] Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA; [7] Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA; [8] Division of Blood and Marrow Transplantation, Stanford University Medical Center, Stanford, CA; [9] Experimental Transplantation and Immunology Branch, National Cancer Institute, Bethesda, MD

Background: Ocular GVHD (o-GVHD) is a known manifestation of chronic GVHD (cGVHD). There are no prospective studies of factors associated with o-GVHD or its impact on quality of life (QOL). We analyzed the above endpoints using the cGVHD consortium database; a prospective multi-center longitudinal observational study.

Methods: The study included patients with cGVHD requiring systemic treatment and enrolled within 3 months of diagnosis. O-GVHD was defined as NIH eye score > 0 and patient reported symptoms (> 1 on 0-10 eye related symptoms or >20 on Lee symptom eye score). Variables associated with o-GVHD at enrollment and subsequent new onset o-GVHD, and the associations with QOL were studied.

Results: The cumulative incidence of o-GVHD at 2 y after cGVHD diagnosis was 58%. Of the 290 patients with o-GVHD, 117 (40%) had it within 3 months of cGVHD diagnosis ("early o-GVHD"). Chronic GVHD characteristics associated with early o-GVHD included: more severe global cGVHD (P<0.001), and greater severity of mouth (P=0.001), esophagus (P=0.002), and liver (P<0.001) involvement. In a multivariable analysis, female sex (OR 2.0, P=0.01) and higher prednisone dose at enrollment (P=0.04) were associated with o-GVHD. Early o-GVHD was not associated with subsequent non-relapse mortality (HR 1.2, P=0.53) or survival (HR 1.1, P=0.85).

Late o-GVHD (new onset > 3 months after cGVHD diagnosis) occurred in 68 patients. The cumulative incidence of late o-GVHD at 2-y post enrollment was 39%. In multivariable modeling, presence of prior grade I-IV aGVHD (HR 1.8, P=0.03) was associated with shorter time to late o-GVHD, while female donor into male recipient (HR 0.5, P=0.05) was associated with longer time to late o-GVHD onset.

The Table shows the association of o-GVHD with QOL metrics, using all available visit data adjusted for center effect, months since enrollment, platelet count, NIH severity, bilirubin, prior aGVHD, and overlap vs. classic cGVHD.

Conclusion: This large multicenter, prospective study shows that o-GVHD affects 58% of patients at 2 y after a diagnosis of cGVHD and is statistically associated with worse QOL and more cGVHD symptoms compared to patients with cGVHD without ocular involvement. Since o-GVHD may be due to permanent destruction of lacrimal glands, prophylactic or pre-emptive clinical trial strategies prior to onset of

QOL Metric	O-GVHD	Estimate	P	NIH Eye score	Estimate	P
FACT-G	No	0*	0.006	0	0*	0.05⁻
	Yes	-2.1		1	-0.7	0.40
				2 or 3	-2.5	0.01
FACT-TOI	No	0*	<0.001	0	0*	0.03⁻
	Yes	-2.6		1	-1.5	0.04
				2 or 3	-2.4	0.01
FACT-BMT	No	0*	0.002	0	0*	0.08⁻
	Yes	-3.0		1	-1.3	0.19
				2 or 3	-3	0.03
Lee Score**	No	0*	<0.001	0	0*	0.01⁻
	Yes	2.8		1	1.7	0.01
				2 or 3	2.1	0.02

⁻overall P value; *Estimate 0-reference category; **Lee symptom score-eye component excluded

irreversible dry eye syndrome are needed. Women, patients on higher doses of prednisone, and those with a history of acute GVHD seem to be at higher risk for o-GVHD.

419

In Silico Derivation of HLA-Specific Alloreactivity Potential from Whole Exome Sequencing of Stem Cell Transplant Donor-Recipient Pairs

Maximilian Jameson-Lee[1], Vishal N. Koparde[2], Juliana K. Sampson[3], Allison F. Scalora[4], Haniya Khalid[5], Nihar Sheth[3], Phil Griffith[1], Myrna G. Serrano[3], Vladimir Lee[5], Catherine H. Roberts[4], Michael C. Neale[6], Gregory A. Buck[3], Masoud Manjili[7], Amir Ahmed Toor[4]. [1] School of Medicine, VCU, Richmond, VA; [2] Bioinformatics, VCU Massey Cancer Center, Richmond, VA; [3] VCU Center for the Study of Biological Complexity, Richmond, VA; [4] Bone Marrow Transplant, VCU Massey Cancer Center, Richmond, VA; [5] VCU Massey Cancer Center, Richmond, VA; [6] Psychiatry, VCU, Richmond, VA; [7] Microbiology and Immunology, VCU Massey Cancer Center, Richmond, VA

Graft vs. host (GVH) effect mediated by donor T cells are responsible for a significant measure of therapeutic benefit as well as toxicity observed following stem cell transplantation (SCT) between HLA-identical donors and recipients. Donor T cell-mediated GVH effects may be influenced by the aggregate alloreactivity to minor histocompatibility antigens (mHA) presented by the HLA in each donor-recipient pair (DRP). The cumulative mHA variation in each DRP may thus be regarded as the alloreactivity potential (AP) of that pair. To estimate AP in DRP, whole exome sequencing (WES) of 4 matched-related (MRD) and 5 unrelated (URD) pairs was performed and revealed extensive coding variation between them. To quantify the contribution of exome sequence variation to AP, data from each DRP was filtered to isolate non-synonymous single nucleotide polymorphisms (SNP) in the GVH direction (polymorphisms present in recipient and absent in donor). Logically, the SNP involved in encoding peptides presented on the HLA in each DRP will contribute to the AP in that pair. To identify these peptides, the nucleotide sequence flanking all of the several thousand SNP in each DRP was obtained with the ANNOVAR software package. All possible resulting nonameric-peptides were interrogated in-silico for their likelihood to be presented by each of the 6 HLA class I molecules in individual DRP, using the Immune-Epitope Database (IEDB) SMM algorithm. The IEDB-SMM algorithm predicted between 1,043,514 and 366,426 peptides/DRP ($\sim$ 18 peptides/SNP). Peptide-HLA binding affinity estimate was reported as an IC50 value, which when <500 nM, predicts that peptides