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Background: High-throughput sequencing (HTS) of antibody gene rearrangements is an emerging tool for minimal residual disease (MRD) monitoring in B cell malignancies in which the malignant clone harbors a monoclonal Ig heavy chain (IgH) and/or light chain (κ or λ) rearrangement. This approach has shown promise in B-ALL and CLL, but application of Ig HTS to clinical multiple myeloma (MM) samples has not been demonstrated previously.

Approach: We conducted HTS of PCR-amplified IgH (VDJ and DJ) and κ/λ (VJ) rearrangements from bone marrow aspirates (BMA) of patients with MM (n=9), MGUS (n=1), and lymphoplasmacytic lymphoma (n=1), and peripheral blood (PB) of a patient with plasma cell leukemia (n=1). In 9/12 samples, an aliquot was enriched for CD138+ cells by immunomagnetic separation and analyzed separately. Dominant clones from enriched and un-enriched aliquots were compared to verify the malignant clonotype sequence. Disease burden in un-enriched samples was also evaluated by microscopy (BMA/PB smear) and ranged from 0 (hemodilute) to 37%.

Results: In 11/12 samples, a clearly dominant IgH and/or κ/λ rearrangement (>2.7% of total sequences, range 2.7-99.9%) was identified with clear separation from background frequency (at least 2.7-fold higher frequency than next most common clone). One sample exhibited an oligoclonal repertoire with no clearly dominant sequence. In 9/9 cases with paired CD138-enriched samples, the dominant sequences in the enriched and un-enriched samples were identical, indicating successful identification of the malignant clonal Ig rearrangements in the un-enriched sample. Results were largely consonant with clinical data, though in one IgG-λ MM sample, no dominant, productive λ rearrangement was detected, and in one IgG-κ MM sample, no dominant, productive heavy chain rearrangement was detected. This may be due to mutations at primer-binding sites in these rearrangements. In both cases, alternative clone-tracking sequences were available from the other loci (i.e., IgH in the first case and κ in the second). In 7/12 cases, >1 dominant sequence among the IgH (VDJ and DJ), κ, and λ rearrangements was identified that would be suitable for longitudinally tracking the malignant clone.

Conclusion: HTS of Ig heavy and light chain rearrangements can successfully identify the MM clone in clinical specimens, including those with low MM burden. Application of this technique to MRD evaluation in MM warrants further development.

79

Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation for Adults with Relapsed and Refractory Mantle Cell Lymphoma: A Single Center Retrospective Analysis in the Rituximab Era

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Backgrounds: Despite recent improvements in the therapy of mantle cell lymphoma, relapsed and refractory disease still portends a dismal prognosis. Allogeneic stem cell transplantation (allo-SCT) represents the only potentially curative therapy in this setting. The aim of this report was to evaluate the results of reduced-intensity-conditioning (RIC) allo-SCT in a retrospective cohort of patients from a single institution.

Patients and Methods: Twenty-nine patients (median age 58 years, range 34-71) undergoing RIC allo-SCT from April 1999 to May 2013 are included in this retrospective analysis. The median number of previous lines of therapy was 5 (range 1-6) with 13 (45%) of patients having previously failed an autologous SCT. Twenty-six patients (90%) had chemosensitive disease at allo-SCT (CR=17, PR=9) and 3 (10%) had stable disease. The second line International Prognostic Index (sIPI) was 0 in 19 patients (65%) and ≥1 in 9 patients (31%). Data was missing in 1. RIC regimens included cyclophosphamide/ fludarabine/ TBI 200cGy with (n=17) or without (n=4) peri-allo-SCT rituximab and melphalan/ fludarabine with (n=6) or without (n=2) alemtuzumab. All patients received unmodified grafts from a matched related (n=12), matched unrelated (n=10) or mismatched unrelated (n=7) donor. Progression-free (PFS) and overall (OS) survival were calculated from the time of allo-SCT. Kaplan-Meier survival curves and a permutation-based logrank test were used to compare PFS and OS based on alemtuzumab use and the sIPI.

Results: All but one patient engrafted with full donor chimerism. The cumulative incidences (CI) of grade II-IV acute GVHD at days +100 and +180 were 36% (95%CI: 19-53%) and 46% (95%CI: 27-64%), respectively. The CI of chronic GVHD at 1 and 2 years was 20% (95%CI: 7-38%) and 29% (95% CI: 12-49%), respectively. The CI of progression of disease and non-relapse mortality at 2 years were 32% (95%CI: 15-51%) and 19% (95%CI: 7-37%), respectively. With a median follow-

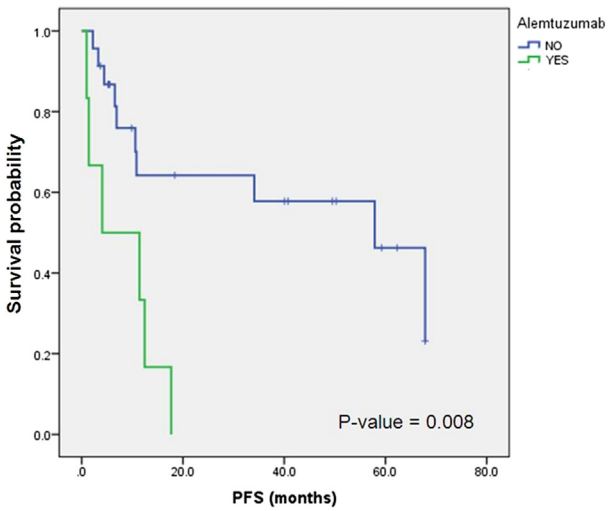

Figure 1. PFS is decreased in patients who receive alemtuzumab

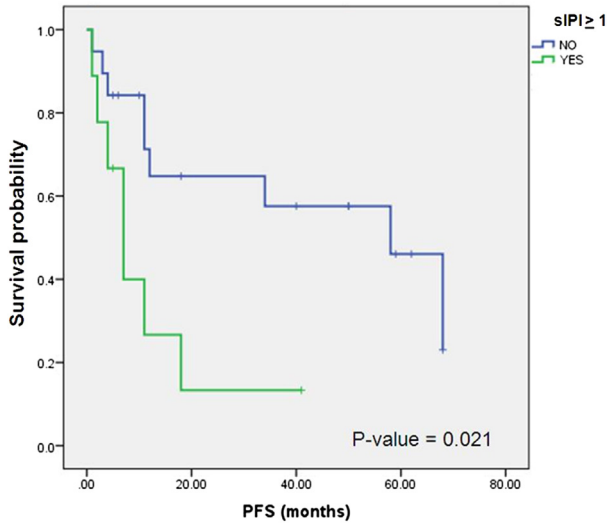

Figure 2. PFS is decreased in patients with sIPI ≥ 1

up in survivors of 40 months (range 2-80 months), the 2-year OS and PFS are 64% (95%CI: 47-86%) and 49% (95%CI: 32-73%), respectively. *In vivo* T cell-depletion with alemtuzumab was associated with a markedly reduced 2-year PFS (0% vs 64%, p=0.008) (Figure 1). Conversely, a sIPI at transplantation <1 was associated with a much improved 2-year PFS (65% vs 13%, p=0.021) compared to higher sIPI values (Figure 2). Similarly, 2-year OS was also significantly reduced with alemtuzumab (33% vs 74%, p=0.016) and in patients with sIPI ≥1 (39% vs 76%, p=0.036).

Conclusions: RIC allo-SCT is a feasible and effective strategy in patients with relapsed and refractory mantle cell lymphoma. High sIPI and use of alemtuzumab in the conditioning regimen are associated with markedly inferior PFS. Higher risk disease and the likely loss of graft-versus-lymphoma with alemtuzumab predict likelihood of failure of RIC allo-SCT in relapsed and refractory MCL patients.

80

Allogeneic Hematopoietic Cell Transplant (Allo-HCT) for Advanced Diffuse Large B-Cell Non-Hodgkin's Lymphoma: The University of Michigan Experience

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The treatment of advanced refractory DLBCL remains a major challenge. High dose therapy with autologous stem cell transplant (HDT/ASCT) has a limited role only in chemo-sensitive relapsed disease, with a curative potential of 25-50%. No therapy has proven to be effective long-term for advanced chemo-refractory DLBCL. Although allo-HCT has curative potential, historically, the outcomes remain unsatisfactory due to high treatment related mortality (TRM) after myeloablative conditioning (MAC), high relapse rate after reduced-intensity conditioning (RIC) regimens and the concern for a less potent graft-versus-lymphoma effect (GVL) A total of 97 DLBCL patients (pts) [M64:F33] undergoing allo-HCT at UM between 1996 and 2011 were retrospectively evaluated. The median age was 52 yrs (range, 23-70). Seventy-four pts (76%) received a MAC [CVB in 49 pts (50%), FluBu4 in 13 pts (13%), others in 12 (12%)]; 23 pts received RIC with FluBu2 (24%); 79 pts (81%) were 8/8 HLA-matched. Only 18 pts (19%) had prior HDT/ASCT. Donor sources were 58 related, 6 mismatched (MM), and 39 unrelated, 12 MM. Stem cell sources were peripheral blood in 89%, bone marrow in 10% and cord blood in 1%. GVHD prophylaxis was tacrolimus/ mini-methotrexate in 71 pts (73%), tacrolimus/ MMF in 16 pts (17%) and others in 10 pts (10%). Fifty-five pts (57%) had chemo-sensitive disease at transplant; 22 pts (23%) had transformed DLBCL.

MAC (n=74)		RIC (n=23)		p-value
Endpoint	**Estimate (95% CI)**	**Endpoint**	**Estimate (95% CI)**	
aGVHD gr 2-4	50% (39%-62%)	aGVHD gr 2-4	39% (22%-63%)	0.49
100 days	54% (43%-66%)	100 days	48% (29%-70%)	
180 days		180 days		
aGVHD gr 3-4	34% (24%-46%)	aGVHD gr 3-4	13% (4%-36%)	0.12
100 days	36% (27%-49%)	100 days	22% (9%-46%)	
180 days		180 days		
cGVHD	32 %(22%-47%)	cGVHD	38% (18%-70%)	0.90
1 year	35 %(24%-49%)	1 year	38% (18%-70%)	
2 years		2 years		
TRM	12% (6%-22%)	TRM	9% (2%-31%)	0.29
100 days	18% (11%-29%)	100 days	22% (9%-46%)	
1 year	23% (15%-35%)	1 year	22% (9%-46%)	
2 years		2 years		
Relapse	33%(23%-45%)	Relapse	43% (26%-67%)	0.84
1 year	33 %(23%-45%)	1 year	43% (26%-67%)	
2 years		2 years		
OS	53% (42%-65%)	OS	39% (24%-65%)	0.29
1 year	43% (33%-56%)	1 year	35% (20%-61%)	
2 years		2 years		
PFS	46% (36%-59%)	PFS	30% (16%-56%)	0.41
1 year	40% (31%-53%)	1 year	30% (16%-56%)	
2 years		2 years		

With a median follow-up time of 12 months (range, 1.6-194), the cumulative incidences of grade 2-4, grade 3-4 acute GVHD, chronic GVHD, TRM, relapse, OS and PFS were not statistically different between the MAC and RIC cohorts (Table). Relapse rate remained high in both groups. Chemosensitivity, donor source and type of DLBCL had no impact on survival.

In summary, allogeneic HCT for advanced DLCBL is feasible, with acceptable TRM. Although, relapse is a major issue, allo-HCT provides these heavily pretreated pts with a platform to combine the GVL effect with other strategies to maximize the potential of long-term disease-free survival.

PEDIATRIC DISORDERS

81

Exposure of Thymoglobulin Is Associated with Overall Survival in Children Receiving Allogeneic-Hematopoietic Cell Transplantation (HCT): Towards Individualized Dosing to Improve Survival

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