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Proceedings from the Second Haploidentical Stem-cell Transplantation Symposium – Haplo2014, San Francisco, California, December 4, 2014

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Abstract

Significant progress has been made over the past decade in haploidentical transplantation with the development of novel methods to control intense alloreactive reactions generated in the major HLA mismatched setting. Application of post-transplantation cyclophosphamide has gained worldwide acceptance as an effective and low cost way to perform this type of transplant, with outcomes now similar with HLA matched unrelated donors. These advances have been made primarily by improving the treatment-related mortality, while disease relapse has emerged as the most common cause of treatment failure. In addition, improvements in immunologic reconstitution after transplant are much needed, not only in haploidentical transplantation but also in all forms of stem cell transplantation. This symposium has focused on some of the most promising methods to

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control alloreactivity in this form of transplantation, application of cellular therapy to prevent disease relapse after transplant, as well as understanding immunologic reconstitution and foreseeable approached to improve immune recovery after transplant.

INTRODUCTION

HLA half-matched related donors are increasingly utilized as source of stem cells due to widespread availability irrespective of race of recipient, lower acquisition cost, fast procurement of stem cells and availability of donors to collect additional cells. Haploidentical transplant outcomes have improved primarily because of the use of post-transplantation cyclophosphamide (PTCy) for GVHD prevention; however, novel methods using partial T cell depletion are equally exciting. As treatment-related mortality (TRM) has decreased with these approaches, prevention of disease relapse has now become the most important target to further improve transplant outcomes. Haploidentical transplantation (HaploSCT) represents an optimal setup to accomplish this due to accessibility to donor cells and the HLA mismatch setting, which may provide enhanced graft-versus-tumor (GVT) effects, if graft-versus-host (GVH) reactions can be controlled. Cellular therapy with T cell subsets or modified T cells may provide an opportunity to tilt the balance of favor of the GVT effect holds the promise to improve relapse rates and transplant outcomes. Improving immunologic reconstitution, remains of paramount importance as represents the key to further decrease toxicity and treatment-related mortality in any form of transplant.

This report summarizes recent developments in haploidentical transplantation presented at the Second Symposium on Haploidentical Transplantation, Haplo2014, held in San Francisco, California. This symposium was organized in 3 sections dedicated to conditioning and graft manipulation, current clinical trials in haploidentical transplantation and to cellular therapy and immunologic reconstitution post-transplant.

The meeting started with an overview presentation by **Dr. Mary Horowitz** on recent CIBMTR trends in use of HLA-matched and alternative donor transplants. First, a growing number of first allogeneic transplants continue to be noted in the US, from approximately 6,000 transplants per year in 2010 to almost 7,500 transplants per year in 2013. The increase in numbers was mostly based on increase in unrelated donor and haploidentical transplants. The 1-year survival in patients with acute leukemia in remission or MDS less than 50 years old using myeloablative conditioning using a matched unrelated donor (MUD) was 70% in 2011. There was steady increase in survival by 8% (95% CI; 7–9%) per year from 1990 until 2011. Since 2009, a growing number of alternative donor transplants were noted with significant increase in haploidentical transplants from 2010 to 2013, from approximately 200 to approximately 400 haploidentical transplants per year. Of 1,646 alternative donor transplants performed in 2010, 41%, 25%, 20%, and 14% used mismatched unrelated, double, single cords and haploidentical donors, while from 1,825 transplants performed in 2013, 43%, 13%, 22%, and 22% used mismatched unrelated, double, single cords and haploidentical donor transplants, respectively. Not unexpected, the use of alternative donor was more pronounced in minority groups (African-American for example) when compared to the Caucasian population.

Historically, in matched unrelated donor transplants a single allele mismatch at HLA-A, -B, -C, or -DRB1 was associated with worse overall survival; this difference disappeared in advanced or high-risk disease [1]. However, such differences do not appear to be the case for haploidentical transplants performed with post-transplant cyclophosphamide, where by using a full haplotype mismatch transplant does not appears to produce higher treatment-related mortality. Moreover, early registry data from CIBMTR comparing outcomes between patients with acute myeloid leukemia receiving a transplant from a haploidentical donor or a MUD showed similar results [2]. Progression-free survival for AML patients at 3 years adjusted for age and disease risk was similar between MUD and haploidentical donor transplants when either myeloablative (50% vs. 45%, HR 0.93, 95% CI 0.7–1.22; p=0.58) or reduced-intensity conditioning/non-myeloablative conditioning was used (44% vs. 46%; HR 1.06, 95% CI 0.79–1.43; p=0.7) [2]

1. Conditioning and Graft Manipulation

Dr. Stefan Ciurea discussed recent developments in haploidentical transplantation performed with PTCy. Several groups reported very good outcomes using PTCy, tacrolimus and mycophenolate mofetil (MMF) as GVHD prevention in this setting and different conditioning regimens [3–9]. In addition, different single-institution studies reported comparative outcomes between haploidentical and HLA matched unrelated donor transplants. Different groups published data on haploidentical transplant outcomes using several conditioning regimens other than the initial one with fludarabine, cyclophosphamide and total body irradiation (Fly/Cy/TBI). While a very low TRM was noted with this regimen, a higher relapse rate (in excess of 50%) seen with this regimen for patients with acute leukemia prompted several groups to explore more intense conditioning regimens for these patients with very good results. Several myeloablative conditioning regimens have now been established as safe and effective including fludarabine with busulfan and thiotepa regimen [5], fludarabine with melphalan and thiotepa or TBI [7], and fludarabine with ablative TBI doses [8]. In these studies, relapse rates for patients with myeloid malignancies varied from 20 to 40% at 1 year. In addition, these groups also compared outcomes of haploidentical transplantation performed with post-transplant cyclophosphamide with HLA matched transplants including matched related and unrelated [7, 10, 11] and found similar transplant outcomes for patients with hematological malignancies between haploidentical and HLA matched transplants. In order to confirm these findings, we did a larger CIBMTR retrospective analysis comparing transplant outcomes in an uniform group of patients with AML who had a transplant with a haploidentical or an 8/8 HLA matched unrelated donor. This study showed almost identical survival at 3 years for patients who received either myeloablative (41% vs. 42%, p=0.87) or reduced-intensity/non-myeloablative conditioning (35% vs. 37%, p=0.89) [2]. These encouraging results suggested that prospective comparative clinical trials are needed to appreciate outcomes between haploidentical transplants performed with PTCy and HLA matched transplants, mostly with MUD transplants, which, in general, take longer time to perform during which patients with more advanced disease may progress and miss the opportunity to receive this life-saving procedure.

Dr. Rupert Handgretinger discussed the evolution of haploidentical transplantation from complete T cells depletion to a partial depletion of alloreactive T cells, and its potential use as a platform to apply post-remission therapy. Depletion of alpha-beta T cells is associated with lower incidence of aGvHD, more rapid immune reconstitution of donor's immune system in the setting of no post-transplantation immunosuppressive therapy. Historically, effective T-cell depletion of mobilized PBSC was based on positive CD34+ selection of pure stem cells developed in the late 1990's. This was found to be associated with higher graft failure rate, infectious complications and TRM, as well as a higher rate of disease relapse [12–14]. In 2003, CD3/19 depletion was introduced as a step forward with the advantage of preserving natural killer (NK) cells in the graft in an attempt to improve relapse rate and possibly rate of infectious complications. In 2011, developments in technique of magnetic cell depletion allowed depletion of alpha-beta T cells, which, in combination with depletion of B cells (TCRαβ/CD19 depletion) had the advantage of retaining all effector cells in the graft, including NKs and γδ T cells, which will be responsible for an enhanced anti-tumor effect and a more rapid immune reconstitution as well. A low NK activity in CD34+ graft was associated with a significant increase in relapse rate including in acute lymphoblastic leukemia (2-year relapse 75 vs. 20%, p=0.01) [15–17]. In haploidentical transplantation, similar results as in matched unrelated donor transplants using KIRs-ligand mismatch donor (e.g. HLA-C1 not homozygous) as well as higher KIR-B content, predicted for a lower relapse rate [18, 19]. Potential role of γδ T cells (after TCRαβ/CD19 depletion) include: lysis of infected or distressed cells, cytokine and chemokine production, regulation of stromal cell function via growth factor production, dendritic cell maturation, and priming of αβ T cells via antigen presentation. In one study, [20] 41 pediatric patients predominantly with hematologic malignancies were treated with αβ T cell depleted haploidentical transplantation using conditioning with fludarabine, melphalan, thiotepa and ATG regimen. When compared with $CD3^+/CD19^+$ depletion and $CD34^+$ selection, patients treated with αβ T cell depletion had much faster recovery of T and NK cells, and patients with acute leukemia had better outcomes. A profound depletion of αβ T cells to 14 $X10^3$/kg was obtained. Patients did not receive any GvHD prophylaxis. The incidence of grade II-IV aGvHD and III-IV aGvHD was 25% and 15%, respectively. Patients recovered γδ T cells in the first month while αβ T and $CD3^+$ cells after 3–4 months post-transplant. $CD3^+$ recovery was rapid with median $CD3^+$ of 290/mcL compared to <50 when $CD3^+/CD19^+$ depleted graft was used. In a preliminary report, 3-year event-free survival for patients with acute leukemia in CR was 66.5%. Dr. Handgretinger also proposed to use αβ TCD haploidentical transplantation as platform to apply post-transplant immunotherapy as no post-transplant immunosuppression is applied after αβ T cell depleted haploidentical transplantation.

Dr. Wing Leung used depletion of naïve $CD45RA^+$ cells as a method to control alloreactivity in haploidentical stem cell transplantation, hypothesizing that depletion of naïve T cells will reduce the risk of GVHD and the preservation of $CD45RO^+$ memory T cells will facilitate engraftment and reduce the risk of infections and relapse post-transplant. The same procedure would also remove B cells to prevent post-transplant lymphoproliferative disease and chronic GVHD. By using an apheresis procedure, a large number of memory cells can be collected from the haploidentical stem cell donors. Preliminary data showed that the $CD3^+CD45RA^+$ cell content in the final apheresis product

was minimal and the TCR alpha beta positive T cells are generally CD45RA negative. The final products contained very few $CD45RA^+$ cells which were $CCR7^+$, $CD27^+$ and $CD31^+$ (recent thymic emigrants). After $CD45RA^+$ depletion, central and effector memory $CD4^+$ cells were retained, as detected by $CCR7^+$, $CD27^+$, and $CD62L^+$ staining. Similarly, most of the $CD8^+$ T cells were effector memory cells which were $CCR7^-$, $CD62L^-$ with heterogeneous expression of CD27. Testing of the immune memory function of T cells showed activity against CMV, EBV, HSV and tetanus toxoid. In the first two months after transplantation, NK cells and $CD8^+$ memory T cells recovered rapidly. Both the $CD4^+$ and $CD8^+$ cell populations had memory phenotypes resembling those in the infused graft. Despite low TREC copy number, a broad TCR repertoire was observed. Based on these preliminary data, a clinical trial is planned to further genetically modify the $CD45RA^-$ cells with chimeric antigen receptors against leukemia associated antigens.

Another emphasis was given to donor selection using high-resolution KIR typing. The hypothesis was that the more alloreactive the donor NK cells, the less complications after transplantation. For example, if the donor NK cells attack the recipient leukemic cells, T cells, viral-infected cells, or dendritic cells, the risk of leukemia relapse, graft rejection, infection progression, and graft-versus-host disease (GVHD), respectively, may be decreased.

Dr. Denis-Claude Roy described the *ex vivo* use of the photosensitizer dibromorhodamine (TH9402) to selectively eliminate alloreactive T cells against host cells resulting in Allo-depleted T-cell Immunotherapeutic (ATIR). Animal studies using fully MHC-mismatched strains have shown that injection of ATIR resulted in the elimination of anti-host T cells and dramatically increased the survival of animals. Preclinical studies using human cells have demonstrated that TH9402 accumulates in activated T cells, but not in resting T cells, and leads to the elimination of anti-host T cells upon visible light exposure (514 nM) as measured using cytotoxic T cell precursor assays. Interestingly, the eradication of activated T cells could be attributed to inhibition of the P-glycoprotein pump. Thus, such a mechanism represents a unique opportunity to destroy cells responsible for graft-versus-host disease, while sparing resting T cells for reactivity against infectious agents and malignant cells. A proliferation assay was recently developed in order to provide rapid assessment of the extent of post-photodepletion (PD) T cell reactivity toward the patient, donor and third party cells. This assay confirmed the ability of PD to eliminate anti-host but preserve anti-third party reactivity as well as CD3/CD28 proliferative response.

A Phase I dose-escalating study has been completed to evaluate the administration of increasing ATIR doses following $CD34^+$ T cell depleted HaploSCT. Patients up to age 62 years with high-risk hematologic malignancies, mostly refractory/relapsed acute myeloid and lymphoid leukemia as well as myelodysplastic syndromes were enrolled. All patients engrafted rapidly (median of 10 days) without any graft failure. Interestingly, there was no severe acute GVHD (grade III-IV) although none of the patients received immune suppressors to prevent GVHD occurrence. Patients administered higher doses of ATIR (0.3 to 5.0×10^6 CD3 cells per kg) had lower TRM and improved survival over those patients with lower ATIR doses (0.1 to 1.3×10^6 CD3 cells per kg) and a control group undergoing

similar transplantation without ATIR support. This effect was mainly attributable to a decrease in infectious complications and low relapse rates.

These findings led to the initiation of a multi-center international Phase II clinical trial (NCT01794299) with ATIR infused at the dose of 2×10^6 CD3/kg. At interim analysis (October 2014), 13 patients were enrolled with median follow-up of 308 days (10 patients had follow up of more than 6 months). PD was found to consistently eliminate activated T cells, both of $CD4^+$ and $CD8^+$ origin, within ATIR cell products while preserving other T cells. Cytotoxic T cell precursors against host cells were also eliminated to very high levels, confirming the quality of ATIR grafts observed in the Phase I study. Patients receiving ATIR did not have severe GVHD, and demonstrate a high overall survival (69 % at 12 months post-transplant based on Kaplan Meier analysis), which is in line with Phase I clinical data. ATIR was concluded to show promising clinical results in $CD34^+$ T cell depleted haplo SCT without any GVHD prophylaxis.

Dr. Xiao-Jun Huang discussed the role of HaploSCT in intermediate and high-risk AML in CR1 with data from Peking University from multicenter study suggesting HaploSCT can achieve comparable outcomes with matched sibling donor (MSD) transplants and superior to chemotherapy alone as post-remission therapy for high-risk acute leukemia patients [21, 22]. Allogeneic hematopoietic stem cell transplantation from an HLA-matched related donor (MRD) or a MUD is recommended by the US National Comprehensive Cancer Network (NCCN) as preferred therapy for patients with intermediate and high risk AML inCR1, according to series of clinical trials and meta-analysis (E3489/S9034, UK MRC AML 10, EORTC/GIMEMA-AML8A). Since HaploSCT plays a more important role in transplantation around the world (the first donor source for allogeneic transplantation in China and more than 10% of allogeneic transplants in Europe), what about the role of HaploSCT in intermediate and high-risk AML in CR1? Data from Peking University and from a multi-center study suggested that HaploSCT can achieve comparable outcomes with MRD transplants and superior to chemotherapy alone as post-remission therapy for high-risk acute leukemia patients, and suggested that HaploSCT may be beneficial both for patients with intermediate and high risk AML in CR1 [21, 22].

For patients with favorable risk factors, would all patients have a favorable outcome? Actually no! The outcomes of AML with core-binding cytogenetic translocation are still not satisfactory. By incorporated allogeneic transplantation as post-remission therapy for high-risk patients, AML05 trial from China demonstrated MRD-directed pre-transplant risk stratification may improve outcomes of t(8;21) AML in CR1. Prospective studies are need to confirm whether allogeneic transplants can improve outcome of high-risk inv (16) or t (16; 16) AML appreciated by continuous detection of MRD after chemotherapy [23].

For patients older than 60 years with intermediate and high-risk AML in CR1, NCCN recommended reduced-intensity conditioning (RIC) transplantation; however, there is still no study compared RIC with full intensity allogeneic transplantation for elderly patients. Micro transplantation (MST) (administration of donor stem cells with chemotherapy in the absence of traditional conditioning chemotherapy) may improve outcomes in this setting and avoid the risk of GVHD in elderly patients with AML in CR1. HaploSCT in older and fit

individuals with myeloablative conditioning (for patients >50 years old, HCT-CI≤2) got similar outcomes compared to younger adults. Whether RIC would be superior to myeloablative conditioning regimens, or how to better evaluate the functional status of elderly patients, or the role of MST compared with traditional transplant procedure for older individuals all remain to be assessed in prospective studies.

In summary, allogeneic transplantation is recommended for patients with intermediate and high-risk AML in CR1, and possible for MRD-stratified high-risk group in patients with favorable cytogenetic risk category. Elderly patients with good performance status (ECOG PS 0–1) may need more aggressive treatment than chemotherapy alone and future studies will determine the best post-remission therapy in these patients.

Dr. Ephraim Fuchs discussed advances in HaploSCT for patients with hemoglobinopathies, especially sickle cell disease (SCD). In this disease, engraftment now is possible in patients with a major HLA mismatch donor without GvHD in more than 50% of patients [24]. This was made possible after introduction of PTCy and rabbit ATG. The goal in this setting was to develop a reduced-intensity regimen that can achieve a stable mixed hematopoietic chimerism state enough to “cure” patients with SCD. The percentage of minimal chimerism needed is not known; however, 20% myeloid chimerism was proposed as enough to maintain higher Hb concentrations (>10g/dL). Other diseases with a high graft failure rate after transplant with the current RIC regimen (Flu/Cy/TBI) were myeloproliferative neoplasm and CLL, in the range of 40–45%. Due to the fairly high graft failure rates, 3 days of ATG have been added at Johns Hopkins to the backbone regimen of the Flu/Cy/TBI regimen with later change of tacrolimus to sirolimus and priming BM donor with G-CSF (which improved harvest cell count from 4.69×10^8/kg to median of 12×10^8/kg). Without adding G-CSF post Day 5, engraftment of neutrophils approached 90% after a median of 25 days, allowing a cure rate of approximately 50% with no treatment-related mortality. Outcomes appeared different when different related donors were used, best with a child (100% engraftment) or brother (75%) as donor. The disadvantage to adding G-CSF priming was increased grade 2–4 aGVHD rate to approximately 50% as compared with approximately 30% historical rates with bone marrow. The next step in overcoming engraftment problems was to abandon the G-CSF priming and increase the TBI dose to 300 or 400 cGy which was not tested yet. Sirolimus-induced tolerance could not be achieved at least in the three patients of that cohort. When it was attempted to be stopped, previously stable mixed chimerism started to deteriorate; however, it was restored after restarting the drug. Dr. Fuchs also discussed the NIH protocol of allogeneic HSCT for patients with SCD using alemtuzumab/TBI-based preparative regimen and PTCy with sirolimus-based GvHD prophylaxis. Engraftment was excellent (100%, n=7) after using 2 days of cyclophosphamide (100mg/m2) and success rates were reported to be approximately 70% (n=5 of 7). Acute GvHD was reported in 1 patient (14%). He concluded with that in patients with SCD engraftment with a haploidentical donor, which is difficult to achieve in this population, is approaching 70%, with low NRM of less than 5% and incidence of aGvHD of less than 15%.

2. Ongoing and Future Clinical Trials in Haploidentical Transplantation

Dr. Ephraim Fuchs discussed the updates of active and planned BMTCTN studies involving haploidentical transplants. BMTCTN1101 is a phase III randomized trial of non-myeloablative conditioning randomizing patients to either double umbilical cord transplant (CBT) versus a haploidentical transplant donor using a bone marrow graft in patients with hematological malignancies. Conditioning for both groups in this study was with the Flu/Cy/TBI regimen and GVHD prophylaxis included high-dose PTCy, MMF, and tacrolimus for haploidentical transplants. The trial opened in mid-2012 with 35 centres activated and 6 more to be activated in the near future. In addition, the German cooperative group (DKMS) has also approved this study as well. At the time of meeting, 133 patients were accrued out of 196 projected (68%) with improved accrual noted in the third quarter of 2014. Other ways to improve accrual to this study was to allow the use of peripheral blood stem cell grafts for haploidentical transplants. Another proposed study was a second phase II myeloablative haploidentical, T cell-replete, unmanipulated bone marrow with PTCy for pediatric and young adult patients with leukemia (PBMTC). In this study, myeloablative preparative regimen will be busulfan-based for myeloid and TBI-based for lymphoblastic diseases. This will be a platform for a possible subsequent randomized study comparing haploidentical and CBT in the setting of myeloablative conditioning. Anticipated enrolment over 2 years will be 31 patients. Results of this study will be compared with MUD transplants reported to CIBMTR. Dr. Fuchs presented the proposed new studies to BMTCTN including CBT vs. HaploSCT for aplastic anemia (CHAT). Primary hypothesis here was that using optimized approaches, alternative donor transplantation for severe aplastic anemia with cord blood and HaploSCT will result in rejection free survival of more than 75% at 1 year. The conditioning proposed for this study was Flu/Cy/TBI regimen. Final study proposal discussed was a phase III randomized study HaploSCT versus “best unrelated donor” HCT. This was proposed as intention to treat with randomization at the time when no MRD was found. Conditioning will be busulfan-, melphalan- or TBI-based for acute leukemia and the Flu/Cy/TBI or FM100/TBI for lymphoma. Uniform GvHD prophylaxis for the haploidentical transplant group will be with PTCy, mycophenolate and tacrolimus with bone marrow (preferred) or PBSC for graft source. This study was postponed primarily because of existing competing protocols.

Dr. Franco Locatelli presented and discussed the results obtained in Rome at the Bambino Gesù Children’s Hospital using the approach of depleting both TCR$\alpha\beta^+$ T cells and $CD19^+$ B cells in HLA-haploidentical HSCT. The incidence of acute and chronic GvHD was very low and no patient died due to these complications. The absence of chronic GvHD has to be valorized in view of the long life-expectancy of pediatric patients. Patients given this type of allograft for a non-malignant disorder have a probability of disease-free survival in the order of 90%, although some patients experienced graft failure and few others died for viral complications [25]. This program offered to the Rome group the opportunity to investigate the recovery of $\gamma\delta$ T cells, which represents the predominant T-cell population in patients during the first weeks after transplantation, being mainly, albeit not only, derived from cells infused with the graft and expanding *in vivo* [26]. This first detailed characterization of $\gamma\delta$ T cells emerging in peripheral blood of children after $\alpha\beta$+ T-cell and CD19+ B-cell depleted HaploSCT also showed that Vδ1 cells are specifically expanded in patients experiencing

cytomegalovirus reactivation and are more cytotoxic compared to those of children who did not experience reactivation. Moreover, the experimental data documented that both Vδ2 and Vδ1 cells display a cytotoxic phenotype and degranulate when challenged with primary acute myeloid and lymphoid leukemia blasts and that Vδ2 cells can be expanded *in vitro* after exposure to zoledronic acid, a drug also rendering primary lymphoid and myeloid blasts more susceptible to the lysis mediated by γδ T cells. While the recovery of innate immunity was prompt with this approach of HaploSCT, recovery of adaptive immunity is still sub-optimal and requires improvement. The add-back of donor T cells expressing a suicide gene is a promising strategy to further improve immune-reconstitution after HaploSCT. Since preliminary interesting results on a chimeric gene incorporating the death domain of inducible caspase 9 (iC9) have been reported in a phase I/II clinical trial conducted in the United States [27, 28], the Rome group has started in November 2014 a phase I/II study enrolling children with either malignant or non-malignant disorders who will receive TCR-αβ/B-cell depleted HaploSCT, followed by the infusion of titrated numbers of iCasp9 T-cells on day 14±4. These iCasp9-modified T-cells can contribute to T-cell immune-reconstitution after T cell-depleted HaploSCT and are eliminated by the administration of a dimerizing molecule, AP1903, if acute GvHD occurs.

With a concern regarding higher incidence of aGvHD and possible high risks associated with this procedure, transplant for older patients with a haploidentical donor has been presumed prohibited. **Dr. Didier Blaise** discussed his group's experience with HaploSCT in patients older than 55 years of age treated with a reduced-intensity regimen. Thirty-one patients over the age of 55 years underwent HaploSCT. Their outcomes were compared in a case-control fashion with age-matched patients transplanted from matched donor (MD) either MRD or MUD. All 3 groups were comparable except for conditioning [70% of patients with MD received NMA conditioning while 100% of HaploSCT patients received a RIC which consisted of fludarabine (5 days), busulfan (2 days), and ATG (2 days)], and GVHD prophylaxis [HaploSCT patients received PTCy with MMF/CsA while matched transplants received conventional GVHD prophylaxis]. All patients engrafted but one in the HaploSCT group (97%, n=30/31). Grade 2–4 aGVHD was not statistically different between haploidentical and MSD transplants; however, was significantly lower when compared with the MUD group. Severe cGVHD was reported to be significantly lower in HaploSCT group when compared to MRD and MUD grafts (0% vs. 16% vs. 14%, p=0.02 and 0.03, respectively). Relapse was similar in the 3 groups. NRM after MUD transplantation was 3-fold higher than after haploidentical or MRD transplantation. The 2-year OS and PFS were significantly better after haploidentical than after MUD transplantation (70 vs. 51% and 67 vs. 38%, p=0.08 and p=0.02; respectively) but did not statistically differ from MRD transplants. Dr. Blaise concluded that T-replete HaploSCT followed by PTCy in patients over 55 years was associated with similar results to MRD transplantation deserving prospective evaluation against HLA matched donors.

Dr. Shin Mineishi reported results of their ongoing protocol using PTCy after myeloablative transplantation using MUD, mismatched MUD (MMUD) and haploidentical donors. Conditioning was busulfan-based in myeloid malignancies and TBI-based in lymphoid malignancies. The source of the graft was peripheral blood stem cells for the great majority

of patients. MMF and tacrolimus were given until days 35 and 100. After a median follow-up of 18 months, a total of 85 patients were enrolled. Overall survival was reported at 64% and 67% in all patients and haploidentical transplants, respectively. Correlative studies on immune reconstitution revealed that recovery of regulatory T-cells (Tregs) was relatively fast, but γδ T-cell reconstitution was very slow, especially in patients who received a transplant from haploidentical donors. γδ T-cells are not HLA-restricted (i.e. they do not cause GVHD) but attack infected cells or with neoplastic transformation, and since γδ T-cells have been shown to improve survival after haploidentical stem cell transplants by decreasing relapse rate without increasing incidence of aGVHD in 153 patients with acute leukemia [20], a clinical trial in haploidentical stem cell transplants has been proposed with infusion of an αβ T-cell depleted donor lymphocyte infusion (DLI) on Day 7 after haploidentical transplantation. Alpha-beta depleted (ABD)-DLI retains the γδ T-cells, NK cells, and additional stem cells, and thus may help engraftment, generate anti-tumor and anti-infection effect, and did not cause higher incidence of GVHD. ABD-DLI products will be composed of more than 1×10^6/kg of γδ T-cells and less than 1×10^5/kg of αβ T-cells. γδ T-cell enrichment ranged from 2.6–11.9% of $CD3^+$ cells (pre-ABD) to above 97% (post-ABD). Using an apheresis procedure 4×10^6 CD34 cell/kg will be collected and infused on transplant Day 0. The remaining product will be αβ deleted and then kept frozen until ready for infusion on Day 7.

Dr. Stefan Ciurea discussed the use of *ex vivo* expanded haploidentical NK cells using membrane-bound IL-21 expressed on surface of antigen presenting cells (APCs) obtained from peripheral blood mononuclear cells (PBMCs) of the same donor as the stem cell donor using a method developed at MD Anderson Cancer Center [29]. A phase I/II clinical trial was initiated (clinicaltrials.gov NCT01904136) using multiple escalating doses of NK cells obtained after 2 weeks of expansion using K562 APCs expressing mbIL-21 to decrease relapse rate post-transplant for patients with myeloid malignancies (AML, MDS, CML). There are several reasons to administer NK cells post-transplant: NK cells generated early post-transplant both in HLA matched and T cell depleted haploidentical transplant are functionally immature with low KIR expression and high NKG2A with reversed $CD56^{bright}$/$CD56^{dim}$ ratio and decreased killing of K562 cells [30–32]. In addition, in retrospective studies, higher NK cell numbers in the first 30–60 days post-transplant period has been associated with decrease relapse rate and increased survival [33, 34]. In haploidentical transplantation with PTCy, the MD Anderson group showed that patients had lowest NK cell numbers and lowest function on day 30 post-transplant. The NK cells had an immature phenotype and low ability to kill K562 and 721.221 cell lines (Denman CJ, et al. NK2013 Meeting; abstract #338). These data provided a strong rationale to administer mature fully functional NK cells in the first month post-transplant. In addition, higher doses obtained from *ex vivo* expansion would enhance anti-tumor effects of the graft. NK cells ware infused on Days −2, +7 and on/after +28 post-transplant. The first infusion was with fresh and the other two were with cryopreserved NK cells. The dose escalation was planned in cohorts of 2 patients starting at 1×10^5/kg up to 1×10^9/kg. Predictive NK alloreactivity or KIR genotyping was not a requirement to participate on study, however, was evaluated in all patients. Three patients were treated to date, one at 1×10^4/kg dose and 2 at 1×10^5/kg. Infusions were not associated with toxicities or the development of aGVHD.

3. Cellular Therapy and Immune Reconstitution

Dr. Massimo Martelli presented updated data using Tregs infused with conventional T cells (Tcons) post "mega-dose" $CD34^+$ selected HaploSCT in 52 patients with high-risk acute leukemia. Median age was 39 years, patients had AML (20 CR1, 17 ≥ CR2), and ALL (10 CR1; 5 in ≥ CR2) treated between 09/2008 and 02/2014. All patients who were transplanted in CR1 were at high risk of relapse. Variable preparative regimens were used, mostly TBI-based (8 Gy in a single fraction with lung shielding), thiotepa (4 mg/kg/day on Days −10 and −9), and fludarabine (40 mg/m^2/day from Day −10 to −6). Forty-eight % (n=25/52) of patients received 35 mg/kg cyclophosphamide from Days −8 and −7 and 44% (n=23/52) patients were given alemtuzumab or thymoglobulin instead 21 days before transplant to prevent interference with Treg-Tcons adoptive immunotherapy. Finally, under the latest protocol 8% (n=4/52) received a reduced dose of cyclophosphamide (30 mg/kg). All patients received donor Tregs (mean 2.5×10^6/kg) on Day −4, which had been immune-selected from a leukapheresis product as previously described [35]. On Day 0 a mean of 9.7×10^6/kg $CD34^+$ cells and 1.1×10^6/kg Tcons were infused. No pharmacological GvHD prophylaxis was given post-transplant. Sustained full- donor engraftment was achieved in the majority of patients 96% (n=50/52). Even though 1.1×10^6/kg ±0.6 Tcons had been infused only 12% (n=6/50) evaluable patients developed ≥ grade II acute GvHD and 2% (n=1/52) patient developed chronic GvHD. There was a rapid, sustained increase in peripheral blood T cell sub-population recovery. $CD4^+$ and $CD8^+$ counts reached 100/μL at a median of days 40 (range 25–150) and 45 (range 18–100) post-transplant.

Compared with T-cell depleted HaploSCT, $CD4^+$ and $CD8^+$ specific for opportunistic pathogens such as *A fumigatus*, *C albicans*, CMV, ADV, HSV, VZV and toxoplasmosis emerged significantly earlier (at each time point $P < 0.0001$).

Overall, at a median follow-up of 4 years (range 7–58 months) the cumulative incidence of TRM was 40% and disease-free survival (DFS) was 58% (n=30/52). In patients receiving anti-T cell antibodies or lower dose of cyclophosphamide, TRM was 23% and DFS was 70%. Only 5% (n=2/41) evaluable patients have relapsed. These patients had evidence of MRD at the time of transplant as they were both NPM+FLT3+ and had received a transplant from non-NK alloreactive donors. Multivariate analysis identified Treg-Tcon adoptive immunotherapy as the only predictive factor associated with a reduced risk of relapse (relative risk 0.06; 95% CI, 0.02–0.35; P=0.02).

Murine mouse models infused with human primary acute myeloid leukemia cells alone or with conventional T cells (Tcons) died of leukemia and GvHD, respectively. Co-infusion of regulatory T cells (Tregs) and Tcons eradicated leukemia without causing GvHD. Human $CD8^+$ T cells harvested from the bone marrow in this last cohort of mice displayed potent alloreactivity against human leukemia, autologous to leukemia PHA blasts and mouse Con A blasts, indicating that Tcons had retained their alloantigen recognition against human and mouse MHC. In contrast, purified $CD8^+$ T cells from spleen and liver displayed no alloreactivity against targets. These data suggested that Tcon homing to the periphery was blocked by Tregs, while Tcons that home to the bone marrow exerted unopposed alloantigen recognition. This phenomenon could be related to the Treg migratory properties, since

CD45RO$^+$ Tregs home to the skin, lungs, and liver but not to bone marrow where CXCR4 expression is too low [36].

It was concluded that Tregs interfered with the pathophysiology of acute GvHD and permitted co-transplantation of enough Tcons to eradicate minimal residual disease, thus eliminating the usual 30–35% incidence of post-transplant high-risk AL relapse without increase in incidence of aGVHD and was associated with improvement in immunologic reconstitution.

Dr. Jeffrey Miller discussed NK cells' unique properties and biology mediating the graft-versus-leukemia effect to protect against relapse. This will hopefully translate into strategies to exploit NK cells for therapeutic purposes. Certain human tumors are more amenable to NK cell based immunotherapy, and the degree of sensitivity to NK mediated killing is often correlated to their expression of ligands for activating NK receptors and not all tumors are targeted through these interactions. Most studies have focused on ways to manipulate the NK cell effectors to decrease the interactions between inhibitory killer-immunoglobulin receptor (KIR) and their MHC ligands. Enthusiasm for this strategy became widespread after the 2002 report from Perugia in which Ruggeri *et al.* published that KIR ligand mismatch between patients and their donors was associated with improved outcomes in myeloid leukemia after T-cell deplete haploidentical transplantation [37]. Further work showed that donors with KIR B haplotypes (typically containing one or more activating KIR) can protect against relapse and prolong survival in AML [38, 39].

To understand the acquisition of NK cell function early after transplant, Dr. Miller reported on the use of 9-color flow cytometry to simultaneously measure both degranulation by CD107a expression (as a surrogate marker for cytotoxicity) and IFNg production by NK cells and their subsets. Patients received either unmanipulated (T cell replete) or potently T cell depleted (CD34$^+$ selected) grafts. Thawed PBMCs were rested overnight in cytokine free media and then incubated with K562 cells to trigger cytotoxicity and cytokine production. PBMCs were stained with CD107a and IFNγ, and then gated on NK cells and NK cell subsets. CD107a degranulation was intact but modestly suppressed (~35%) at 3 months after both T cell deplete and T cell replete HSCT with further recovery of killing at 6 months. By contrast, at 3 months after T cell repleted HSCT there was potent and sustained suppression of IFNγ production by CD56$^+$ cells. The cohort of patients receiving T cell depleted (CD34-selected) grafts without immunosuppression also exhibited significant suppression of IFNγ at 3 and 6 months after transplantation. Strategies that improve on this function have the potential to decrease relapse.

Another method to deliver alloreactive NK cells to the patient involves adoptive transfer of donor NK cells enriched *ex vivo* and infused into the recipient. These NK cells are presumed to be mature and fully functional and educated in the donor. The first trial of this approach was conducted at the University of Minnesota. Patients with metastatic melanoma, metastatic renal cell carcinoma, or poor prognosis AML were enrolled in the trial. PBMCs were collected from haploidentical related donors and CD3 depleted (now CD3 and CD19 depleted) before being incubated overnight in IL-2. Prior to NK cell infusion, patients underwent a preparative regimen that involved three different chemotherapy preps: high-

dose cyclophosphamide and fludarabine (Hi-Cy/Flu), low-dose cyclophosphamide and methylprednisone or fludarabine. Following infusion patients received IL-2 daily for 14 days. NK cell expansion was only observed for patients receiving the preparatory regimen of Hi-Cy/Flu. Successful expansion of NK cells was determined by the detection of greater than 100 NK cells/uL of blood 12–14 days after infusion. On this protocol 30% of poor prognosis AML patients achieved a CR. Higher rates can be achieved when combined with IL-2 diphtheria toxin fusions intended to deplete regulatory T cells (Treg) [40]. Long-term disease-free survival was achieved only when this was followed by allogeneic transplantation.

While Dr. Miller's group and others have shown that IL-15 is necessary for homeostasis of $CD8^+$ T and NK cells [40, 41], approaches to apply this clinically are complicated. It is believed that endogenous IL-15 in serum binds to IL-15Rα to form a natural complex. This natural complex interacts with IL-2Rβγ on NK cells and $CD8^+$ T cells through a process called IL-15 trans-presentation [42, 43]. Monocytes and dendritic cells express the highest density of IL-15Rα [44]. There are several IL-15 products in clinical development. Each has unique structural differences that determine how they interact with NK cells in vivo. The recombinant human (rh) IL-15 produced by Steven Creekmore's group at the NCI in its unbound form (monomeric) was used to in vivo expand adoptively transferred NK cells. First in human trial treating post-transplant relapse using IL-15/IL-15Rα-Fc super agonist complexes (Altor Biosciences) is ongoing, the design of which includes a mutant IL-15, the addition of a sushi domain to inhibit complement activation, increased avidity of the molecule to IL-2R βγ on NK cells, and increased half life and stability by inclusion of the Fc domain [45–47].

The novel concept of NK cell memory has emerged over the past several years with the identification of subsets of NK cells in mice that mount heightened secondary responses in an antigen-specific fashion in models cytomegalovirus infection [48, 49]. In humans, $CD57^+NKG2C^+$ NK cells specifically expand in response to human CMV [50–52] and referred to as adaptive NK cells [53]. Those "adaptive" NK cells produced significantly more IFN-γ and TNF in response to IgG-coated S2 insect cells (an established assay for measuring ADCC activity), but degranulation is similar to conventional NK cell subsets, reflecting the known lower activation threshold for degranulation relative to cytokine production in NK cells. Thus, "adaptive" NK cells appear to be specialized for enhanced target recognition through CD16 for not only CMV infection [54] but also against tumor targets. It is believe these cells are optimally primed by CMV to change the repertoire of NK cells to fight cancer.

Another direction was to develop strategies to bind NK cells to target antigens by development of bi- and tri-specific killer engagers (BiKEs and TriKEs; fusions of the scFv of anti-CD16 with one or more target Ag) [55, 56]. It has been shown that anti-CD16×33 BiKE activation overcomes inhibitory signaling via class I HLA to potently kill primary cancer targets [57], as well as targeting $CD33^+$ myeloid derived suppressor cells (MDSC) [58].

Dr. Carl June discussed strategies for consolidation therapies after allogeneic transplantation using genetically engineered T cells. A supporting rationale for this approach is that there's an emerging long-term safety profile following the infusion of genetically engineered T cells in humans. At present, there are more than 1000 patient-years of safety observed to date in various trials with genetically modified T cells in patients with HIV and various forms of hematologic malignancies [59]. Importantly, there have been no incidences of transformation or genotoxicity following gene-modified T cell infusions.

Ex vivo expanded allogeneic T cells have been infused into recipients of allogeneic HCT in previous years. It appears that there has been less graft-versus-host disease than would be expected after donor leukocyte infusion [60], even after anti-CD3 and CD28 activation in vitro [61, 62]. The mechanism for this relative safety remains unknown but it may be related in part, to the depletion of antigen presenting cells in the infused donor leukocyte preparation.

Investigators at the National Cancer Institute carried out the first trial of chimeric antigen receptor (CAR) T cell infusions manufactured from the donors of patients who had relapsed after previous alloHSCT and prior donor leukocyte infusions from the original donor [63]. In this phase one study, 10 patients were given infusions of CD19 CAR T cells manufactured from each patient's original HSCT donor; in six cases from matched sibling donor and in four cases the donor was unrelated. There were no significant cases of graft versus host disease. There was some evidence of antitumor activity with one patient with refractory CLL achieving a CR and one PR in a patient with mantle cell lymphoma.

In the ongoing trial with CTL019 CAR T cells at Children's Hospital of Philadelphia, both autologous and allogeneic CAR T cells manufactured from a chimeric recipient have been infused [64]. In updated results (July 2015) a total of 53 children and young adults with CD19+ALL, median age 11 years [range= 4–24] have been infused with a median of 4.3 $\times 10^6$ CTL019 cells/kg [range= 1–17.4 $\times 10^6$ /kg]. In 35 of the 53 patients, the T cells were collected from patients who had relapsed after alloHSCT and in all cases were 100% donor in origin. No cases of acute or chronic GVHD have been observed. The response rate did not differ whether autologous or allogeneic CAR T cells were infused, as 50 pts (94%) achieved a CR. All but 5 patients developed grade 1 to 4 cytokine release syndrome at the peak of CAR T cell expansion, and there was no observable difference in the toxicity as to whether the CTL019 T cells were autologous or allogeneic in origin. Together these promising results suggest that the use of universal CAR T cells manufactured from donors or even 3rd party donors could be feasible and have anti-leukemic efficacy.

Dr. Hui-Sheng Ai discussed the concept and clinical investigation of microtransplantation which consists of utilizing a conditioning regimen which contains either chemotherapeutics (such as cytarabine in AML) and/or targeted therapy (such as Decitabine in MDS) to at least partially eliminate leukemia cells but avoids immunosuppressive agents (such as TBI, ATG, and fludarabine) to preserve recipient immune function. This is followed by programmed infusion of G-CSF mobilized allogeneic peripheral blood stem cells (HLA partially-matched or fully-mismatched, related or unrelated donor) without any GVHD prevention. Persistent donor microchimerism instead of full or mixed chimerism is presumed to be present; graft-

versus-leukemia (GVL) and recipient-versus-leukemia (RVL) effects are induced with avoidance of clinical acute GVHD [65]. An updated data on microtransplantation for treating elderly AML patients was reported. One hundred and three AML patients ages 60–88 years were assigned to receive induction chemotherapy with mitoxantrone and cytarabine with (n=75) or without (n=28) G-CSF mobilized HLA-mismatched peripheral blood stem cell (G-PBSCs) infusion. Patients who achieved complete remission received 2 other cycles of consolidation with intermediate dose cytarabine with or without G-PBSCs. The complete remission rate was 76% in the G-PBSC group compared with 42.8% in the chemotherapy group. Median time to neutrophil count recovery was (11.5 vs.16 days) and platelet count recovery was (15.5 vs. 20 days) after G- PBSC and chemotherapy, respectively. Treatment-related death rate was 9.3% in the G-PBSC group compared with 14.3% in the chemotherapy group. No GVHD was observed in any patient.

Another multi-center study of microtransplantation as consolidation in adult AML was also recently reported. In this study, 101 patients with AML in the first remission received programmed infusions of G-PBSCs after each of three cycles of high dose cytarabine conditioning without GVHD prophylaxis. The median numbers of mononuclear, $CD34^+$ and $CD3^+$ cells infused per course were 2.8×10^8/kg, 1.8×10^6/kg, and 1.1×10^8/kg, respectively. Patients who received a high dose of donor $CD3^+$ T cells ($\geq1.1\times10^8$/kg) with each infusion had a higher 6-year LFS (76.4% vs. 49.5%) and OS (82.1% vs. 55.3%) when compared to those receiving a lower dose ($< 1.1\times10^8$/kg) of donor $CD3^+$ T cells. No GVHD was observed in any patient. Donor microchimerism was observed in female patients who were available for Y chromosome analysis in both studies. Microtransplantation speeded up hematopoietic recovery, improved complete remission rates and survival. This appeared to separate GVL from GVHD and was done across major HLA barrier. An open, randomized, controlled and international multi-center clinical study on microtransplantation for the treatment of *de novo* elderly AML (IMCG- EAML2014) is ongoing to further validate the efficacy of this approach. Microtransplantation as a novel strategy for treating other malignancies such as MDS, ALL, lymphoma, multiple myeloma and some solid tumors is under investigation.

Dr. Leo Luznik discussed possible mechanisms of PTCy as a method to promote induction of immunological tolerance for GvHD prevention in the context of allogeneic HSCT [66, 67]. He then briefly reviewed data suggesting favorable immune reconstitution marked by low incidence of invasive viral infections and Epstein-Barr virus (EBV)-related post-transplantation lymphoproliferative disease (PTLD) when PTCy was used to prevent GVHD [68]. Dr. Luznik acknowledged that the *in vivo* mechanisms of PTCy and particularly of the immune dynamics occurring during the first year after allogeneic HSCT using PTCy remain poorly understood and that there is limited data regarding immune reconstitution after allogeneic HSCT utilizing PTCy. He then went on to present the recent data suggesting that immune reconstitution in the first 1–2 months after PTCy is characterized by persistence of activated regulatory T cells (Tregs) [69]. Dr. Luznik discussed how donor Tregs cells in both mouse and human models of HSCT are resistant to PTCy-induced cytotoxicity owing to increased expression of Aldehyde dehydrogenase (AD), the enzyme primarily responsible for *in vivo* detoxification of cyclophosphamide, upon allogeneic stimulation in a

lymphopenic environment [70, 71]. Finally, Dr. Luznik presented data on the assessment of immune reconstitution in 71 patients undergoing MAC HaploSCT with PTCy (50mg/kg on days +3 and +4), MMF (administered on Days +5 to +35), and tacrolimus (administered on Days +5 to +180) as GVHD prophylaxis and 73 patients undergoing MAC HLA-matched allogeneic HSCT with PTCy (50mg/kg on days +3 and +4) as sole GVHD prophylaxis. Flow cytometry-based immune-phenotyping was performed on peripheral blood samples collected serially at predetermined time points. In all groups, NK cells recovered to normal donor counts by 6 months. In patients without GVHD, NK recovery was more rapid with no significant difference by 2–3 months. By 1 year following HaploSCT and HLA-matched HSCT, median absolute lymphocyte counts (ALCs) were in the normal range (1,100–4,800 cells/μl) and B-cell counts were higher than those in normal donors. Results were similar after HLA-matched and HaploSCT. However, $CD4^+$ and $CD8^+$ T-cell counts at 1 month were statistically significantly lower after HaploSCT ($p<0.0001$). Median $CD4^+$ T-cell counts at 1 year were significantly lower after both HLA-matched and HaploSCT compared with normal donors. At 6 months and 1 year post-transplant there was no significant difference in $CD8^+$ T- cells after HaploSCT or HLA-matched HSCT compared with normal donors. There was a trend towards lower total $CD8^+$ T-cell counts at all time-points HaploSCT compared with HLA- matched HSCT. Phenotypic effector memory (EM) and terminally differentiated effector memory T-cells (TEMRAs) recovered rapidly after HSCT, particularly within the $CD8^+$ fraction after both HaploSCT and HLA-matched HSCT. However, phenotypically naïve cells remained low throughout the first post-HSCT year. Overall these preliminary data suggested that after PTCy-based GVHD prophylaxis comparable reconstitution of NK and B-cells; however $CD4^+$ and $CD8^+$ T-cell early recovery lags after HaploSCT when compared to HLA-matched HSCT. This delay is likely attributable to the addition of MMF and tacrolimus, which may be mitigated by discontinuation of MMF at Day 35, resulting in equivalent $CD4^+$ T-cell and $CD8^+$ T-cell numbers by 3 and 6 months respectively. Dr. Luznik concluded his presentation by indicating that ongoing work with next-generation sequencing (NGS) will help further decipher the T-cell repertoire reconstitution post HSCT with PTCy, particularly the effect of clinical variables on its diversity.

Dr. Enrico Lugli discussed cellular mechanisms of T cell reconstitution following haploidentical transplantation early after T-replete transplants with PTCy which depends on the persistence and function of adoptively-transferred T cells. PTCy is thought to preferentially target proliferating T cells that are activated *in vivo* following recognition of alloantigen, although evidence in humans remains elusive. The optimal scenario in this setting would be to spare non-alloreactive donor naïve and memory T cells, both to guarantee primary responses to newly encountered antigens and, simultaneously, to confer adaptive immunity to the recipient. Cutting-edge 18-color flow cytometry, antigen specific assays and T cell receptor sequencing allowed to track T cell dynamics during the early days and weeks following T replete haploidentical transplantation in relapsed lymphoma patients [11] and determined the effect of PTCy on T cell subsets adoptively-transferred from the bone marrow [72]. Fine analysis of T cell differentiation combined with activation markers revealed that, at Day +3, before the administration of PTCy, approximately 25% of $CD4^+$ and 60% of $CD8^+$ memory/effector-phenotype T cells preferentially expressed the

proliferation marker Ki-67, a surrogate indicator of PTCy susceptibility. Conversely, naïve T (T_N) cells were almost negative for this marker. The Ki-67$^+$, PTCy susceptible cells, derived from both the T_N and memory T cell compartments, as revealed by incubation of highly-purified T_N and memory T cells with allogeneic antigen-presenting cells. Strikingly, at Day +7, early after PTCy administration, the circulating T cell compartment was mostly enriched in CD45RO$^-$CCR7$^+$CD27$^+$CD95$^+$ T stem cell memory (T_{SCM}), a recently-discovered memory T cell subset endowed with superior reconstitution capacity in preclinical models [73, 74]. Vice versa, *bona fide* CD95$^-$ T_N cells were absent, thereby suggesting that T_{SCM} derived from T_N cells that survived PTCy *in vivo*. Post-transplant T_{SCM}-phenotype cells displayed a pattern of effector cytokine production mostly similar to naturally-occurring T_{SCM} cells from healthy donors, and vigorously proliferated in response to IL-15, hence confirming the acquisition of memory properties *in vivo*.

Self/tumor-associated antigen (TAA)-specific T cells are mostly T_N in healthy donors: the acquisition of memory/effector phenotypes by these cells in the recipient would suggest they progressed through an early T_{SCM} stage in the post-transplant environment. CD8$^+$ T cells specific for MART1 and Wilm's tumor (WT)-1 epitopes could be detected in patients up to 90 days post-HSCT and expressed memory/effector markers. Similarly, CD8$^+$ T_N cells from CMV$^-$ donors were able to mount CMV-specific responses when transferred in CMV$^+$ recipients. Differently, adoptively-transferred pathogen-specific memory T cells were able to expand in the recipient only in the presence of the cognate antigen. Indeed, donor CMV-specific T cells (in the context of CMV$^{+/-}$ transplants) as well as Flu-specific T cells were undetectable in the circulation. However, the former were readily measurable in the peripheral blood of CMV$^+$ patients, and shared clonal relationship with those that were transferred with the graft.

Collectively, these data shed light on the basic immunological mechanisms governing PTCy function *in vivo* and indicate that transferred T_N may acquire T_{SCM} traits in the lymphopenic patient and subsequently contribute to immune reconstitution by generating effector cells. In the context of antigen-specific T cell responses, PTCy effectively targets alloreactive T cells *in vivo* while sparing bystander pathogen and self/TAA-specific T cells. Indeed, primary and memory T cell responses can be generated despite PTCy even in the presence of persistent antigen in the host. Nevertheless, the depletion of Ki-67$^+$ effector/memory phenotype cells by PTCy may deplete some pathogen-specific clones and thus explain the increased susceptibility of these individuals to post-transplant infectious complications.

Dr. Marcel van den Brink discussed strategies to enhance post-transplant T cell reconstitution. T cell deficiency after allogeneic transplantation remains an issue 1–2 years post-transplant, this will cause increase in risk of infectious complications, especially viral and fungal infections, as well as risk of relapse of disease. It has been shown that recovery of T cell function in younger patients is faster than in older patients; this was probably a result of thymic dysfunction in aging population. Other factors associated with thymic dysfunction include: conditioning (i.e. chemotherapy, radiation and the use of antibodies) and GvHD. Several strategies to enhance T cell recovery following allogeneic HSCT, which are currently in trials or in development, were discussed, including: interleukin-7, keratinocyte growth factor (KGF), sex steroid inhibition, and administration of T cell precursors.

Some of the non-hematopoietic stromal cells that support thymopoiesis include fibroblast, epithelial (TEC) and endothelial cells. This support is done though important thymopoietic factors involved in T cell commitment like Notch ligand DLL1 and DLL4, migration like CCL21, CXCL12, and CCL25, as well as proliferation cytokines like SCF, IL-7 and IL-15. The thymus is exquisitely sensitive to negative stimuli (i.e. stress, infection, chemotherapy, radiation therapy, and corticosteroids use). Poor thymus function leads to reduced output of naïve T cells which, in turn, leads to reduced T cell receptor diversity, poor response to new infections and malignant relapse. It has been shown that sex steroid ablation promotes production of lymphoid progenitors in the bone marrow, restores thymic architecture and T cell development, and increases thymic export and diversity of T cell receptor repertoire [75–78]. Dr. Van den Brink elegantly showed that sex steroids modulate some key thymopoietic factors include delta like ligand 4 (DLL4). Targeting LHRH-receptor promotes thymic re-growth through enhancement of DLL4 expression, subsequently enhances immune recovery after acute thymic damage and peripheral T cell function. This was seen in both genders.

Interleukin-22 (IL-22) was also found to be another important factor in thymopoiesis. It is secreted by lymphoid tissue and is associated with maintenance of barrier function and induction of innate antimicrobial molecules at mucosal surfaces [79–82]. Dr. van den Brink showed that administration of recombinant IL-22 can rapidly boost thymopoiesis after TBI, promotes an increased number of both thymocytes and TECs, and enhances T cell development during GvHD through increased thymopoiesis and exporting of more naïve T cell to the circulation. This is being translated to a phase I trial using human IL-22-Fc in combination with steroids in treatment of aGvHD.

In summary, outcomes of haploidentical transplants have improved, now approaching outcomes of HLA matched transplants. Haploidentical transplants are an area of active investigation in transplantation with novel approaches focused on better controlling of alloreactive reactions, elimination of post-transplant immunosuppression, prevention of disease relapse and improving in immunologic reconstitution. Multiple exciting clinical trials are ongoing will likely further advance our knowledge and improve outcomes of patients treated with haploidentical donors over the next several years.

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KEY POINTS:

- Several methods have been developed to perform haploidentical transplantation
- Post-transplant cyclophosphamide has been associated with lower treatment-related mortality and outcomes comparable with HLA matched transplants
- Future directions will explore decrease relapse rate and improving immunologic reconstitution post-transplant