Title

Proceedings from the Second Haploidentical Stem Cell Transplantation Symposium—Haplo2014, San Francisco, California, December 4, 2014

Journal

Transplantation and Cellular Therapy, 22(4)

ISSN

2666-6375

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Publication Date

2016-04-01

DOI

10.1016/j.bbmt.2016.01.001

Biol Blood Marrow Transplant 22 (2016) 594–604

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Report

Proceedings from the Second Haploidentical Stem Cell Transplantation Symposium—Haplo2014, San Francisco, California, December 4, 2014

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Article history:
Received 31 December 2015
Accepted 6 January 2016

Key Words:
Haploidentical transplantation
Post-transplantation cyclophosphamide
T cell depleted
Immunologic reconstitution after transplantation
Cellular therapy

A B S T R A C T

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 transplantation, with outcomes now similar to those from HLA-matched unrelated donors. These advances have resulted in improved treatment-related mortality, whereas disease relapse has emerged as the most common cause of treatment failure. In addition, improvements in immunologic reconstitution after transplantation are much needed, not only in haploidentical transplantation but in all forms of stem cell transplantation. This symposium has focused on some of the most promising methods to control alloreactivity in this form of transplantation and application of cellular therapy to prevent disease relapse after transplantation, as well as understanding immunologic reconstitution and foreseeable approaches to improve immune recovery after transplantation.

INTRODUCTION

HLA–half-matched related donors are increasingly utilized as source of stem cells because of lower acquisition cost, widespread availability regardless of race of recipient, fast procurement of stem cells, and availability of donors to collect additional cells. Haploidentical transplantation outcomes have improved primarily because of the use of

Financial disclosure: See Acknowledgments on page 603.

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http://dx.doi.org/10.1016/j.bbmt.2016.01.001
1083-8791/

post-transplantation cyclophosphamide (PTCy) for graft-versus-host disease (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 transplantation outcomes. Haploidentical transplantation (HaploSCT) represents an optimal setup to accomplish this because of accessibility to donor cells and because the HLA-mismatch setting may provide enhanced graft-versus-tumor effects if graft-versus-host 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 graft-versus-tumor effect and holds promise to improve relapse rates and transplantation outcomes. Improving immunologic reconstitution remains of paramount importance, as it represents the key to further decreasing toxicity and TRM in any form of transplantation.

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

The meeting started with an overview presentation by Dr. Mary Horowitz on recent Center for International Blood and Marrow Transplant Research (CIBMTR) trends in the use of HLA-matched and alternative donor transplantation. First, a growing number of first allogeneic transplantations continues to be noted in the United States, from approximately 6000 transplantations in 2010 to almost 7500 transplantations in 2013. The increase was mostly based on increase in unrelated donor and haploidentical transplantations. The 1-year survival in patients with acute leukemia in remission or myelodysplastic syndrome (MDS) younger than 50 years old using myeloablative conditioning with matched unrelated donor (MUD) was 70% in 2011. There was steady increase in survival of 8% (95% confidence interval [CI], 7% to 9%) per year from 1990 until 2011. Since 2009, a growing number of alternative donor transplantations was noted, with a significant increase in haploidentical transplantations of approximately 200 in 2010 to approximately 400 in 2013. Of 1646 alternative donor transplantations performed in 2010, 41%, 25%, 20%, and 14% used mismatched unrelated, double-unit cord blood, single-unit cord blood, and haploidentical donors, whereas from 1825 transplantations performed in 2013, 43%, 13%, 22%, and 22% used mismatched unrelated, double-unit cord blood, single-unit cord blood, and haploidentical donor transplantations, respectively. Not unexpectedly, the use of an alternative donor was more pronounced in minority groups (African-American for example) than in the Caucasian population.

Historically, in MUD transplantations, 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 transplantations performed with PTCy, where using a full haplotype–mismatched transplant does not appear to produce higher TRM. Moreover, early registry data from CIBMTR comparing outcomes between patients with acute myeloid leukemia (AML) 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 transplantations when either myeloablative (50% versus 45%; hazard ratio, .93; 95% CI, .7 to 1.22; $P = .58$) or reduced-intensity conditioning (RIC)/nonmyeloablative conditioning was used (44% versus 46%; hazard ratio, 1.06; 95% CI, .79 to 1.43; $P = .70$) [2].

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 with different conditioning regimens [3-9]. In addition, different single-institution studies reported comparative outcomes between haploidentical and HLA MUD transplantations. Different groups published data on haploidentical transplantation outcomes using several conditioning regimens other than fludarabine (Flu), cyclophosphamide (Cy), and total body irradiation (TBI). Although very low TRM was noted with this regimen, a higher relapse rate (in excess of 50%) 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 Flu with busulfan and thiotepa [5], Flu with melphalan and thiotepa or TBI [7], and Flu 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 PTCy with HLA-matched transplantations (including matched related and unrelated) [7,10,11], and found similar transplantation outcomes for patients with hematological malignancies who had haploidentical and HLA-matched transplantations. To confirm these findings, we did a larger CIBMTR retrospective analysis comparing transplantation outcomes in an uniform group of patients with AML who had transplantation with a haploidentical or an 8/8 HLA MUD. This study showed almost identical survival at 3 years for patients who received either myeloablative (41% versus 42%, $P = .87$) or RIC/nonmyeloablative conditioning (35% versus 37%, $P = .89$) [2]. These encouraging results suggested that prospective comparative clinical trials are needed to appreciate outcomes between haploidentical transplantations performed with PTCy and HLA-matched transplantations, mostly with MUD transplantations, 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 cell 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 acute GVHD (aGVHD) and more rapid immune reconstitution of donor's immune system in the setting of no post-transplantation immunosuppressive therapy. Historically, effective T cell depletion of mobilize peripheral blood stem cells (PBSC) was based on positive $CD34^+$ selection of pure stem cells developed in the late 1990s. This was found to be associated with higher rates of graft failure, 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, the rate of infectious complications. In 2011, developments in technique of magnetic cell depletion allowed depletion of αβ T cells, which, in combination with depletion of B cells (TCR αβ/CD19 depletion) had the advantage of retaining all effector cells in the graft, including NKs and γδ T cells, which are responsible for an enhanced antitumor effect and a more rapid immune reconstitution, as well. Low NK activity in a $CD34^+$ graft was associated with a significant increase in the relapse rate, including in acute lymphoblastic leukemia (ALL) (2-year relapse, 75% versus 20%; $P = .01$) [15-17]. In haploidentical transplantation, similar results as in MUD transplantations using KIR-ligand mismatch donor (eg, HLA-C1 not homozygous) as well as higher killer immunoglobulin receptor (KIR) B content predicted a lower relapse rate [18,19]. The potential role of γδ T cells (after TCRαβ/CD19 depletion) includes 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 1 study [20], 41 pediatric patients, predominantly with hematologic malignancies, were treated with αβ T cell–depleted haploidentical transplantation using conditioning with Flu, melphalan, thiotepa and antithymocyte globulin (ATG). When compared with $CD3^+/CD19^+$ depletion and $CD34^+$ selection, αβ T cell depletion led to much faster recovery of T and NK cells, and patients with acute leukemia had better outcomes. A profound depletion of αβ T cells to 14×10^3/kg was obtained. Patients did not receive any GVHD prophylaxis. The incidences of grades II to IV aGVHD and III and IV aGVHD were 25% and 15%, respectively. Patient's γδ T cells recovered in the first month, whereas αβ T and $CD3^+$ cells were recovered more than 3 to 4 months after transplantation. $CD3^+$ recovery was rapid with median $CD3^+$ of 290/μL, compared with <50 when a $CD3^+/CD19^+$–depleted graft was used. In a preliminary report, 3-year event-free survival for patients with acute leukemia in complete remission (CR) was 66.5%. Dr. Handgretinger also proposed to use αβ T cell–depleted haploidentical transplantation as a platform to apply post-transplantation immunotherapy, as no post-transplantation 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 that preservation of $CD45RO^+$ memory T cells will facilitate engraftment and reduce the risk of infections and relapse after transplantation. The same procedure would also remove B cells to prevent post-transplantation lymphoproliferative disease and chronic GVHD (cGVHD). By apheresis, 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 αβ-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 $CCR7^-$ and $CD62L^-$ with heterogeneous expression of CD27. Testing of the immune memory function of T cells showed activity against cytomegalovirus (CMV), Epstein-Barr virus, herpes simplex virus, and tetanus toxoid. In the first 2 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 a low TREC (T-cell receptor excision circle) 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.

Emphasis was also given to donor selection using high-resolution KIR typing. The hypothesis was that the more alloreactive the donor NK cells, the fewer 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 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 allogeneic-depleted T cell immunotherapeutic (ATIR). Animal studies using fully MHC-mismatched strains have shown that injection of ATIR resulted in the elimination of antihost 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 antihost 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 GVHD while sparing resting T cells for reactivity against infectious agents and malignant cells. A proliferation assay was recently developed 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 antihost but preserve antithird-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 after $CD34^+$ T cell–depleted HaploSCT. Patients up to age 62 years with high-risk hematologic malignancies, mostly refractory/relapsed AML and ALL as well as MDS were enrolled. All patients engrafted rapidly (median, 10 days) without any graft failure. Interestingly, there was no severe acute GVHD (grades III and V), although none of the patients received immune suppressors to prevent GVHD occurrence. Patients administered higher doses of ATIR (.3 to 5.0×10^6 CD3 cells/kg) had lower TRM and improved survival over those patients with lower ATIR doses (.1 to 1.3×10^6 CD3 cells/kg) and a control group undergoing similar transplantation without ATIR support. This effect was mainly attributed to a decrease in infectious complications and low relapse rates.

These findings led to the initiation of a multicenter 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 after transplantation 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 HaploSCT 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 outcomes comparable to matched sibling donor transplantations and superior to chemotherapy alone as postremission 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 as preferred therapy for patients with intermediate- and high-risk AML in CR1, according to series of clinical trials and meta-analysis (E3489/S9034, UK MRC AML 10, EORTC/GIMEMA-AML8A). Because 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 transplantations in Europe), what about the role of HaploSCT in intermediate- and high-risk AML in CR1? Data from Peking University and from a multicenter study suggest that HaploSCT can achieve outcomes comparable with MRD transplantations and superior to chemotherapy alone as postremission 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 postremission therapy for high-risk patients, the AML05 trial from China demonstrated minimal residual disease–directed pretransplantation risk stratification may improve outcomes of t(8;21) AML in CR1. Prospective studies are need to confirm whether allogeneic transplantations can improve outcome of high-risk inv (16) or t (16; 16) AML appreciated by continuous detection of minimal residual disease after chemotherapy [23].

For patients older than 60 years with intermediate- and high-risk AML in CR1, the US National Comprehensive Cancer Network recommends RIC transplantation; however, to our knowledge, no study has compared RIC with full-intensity allogeneic transplantation for elderly patients. Microtransplantation (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, hematopoietic cell transplantation–specific comorbidity index $\leq$ 2) achieved similar outcomes as younger adults [83]. Whether RIC would be superior to myeloablative conditioning regimens, how to better evaluate the functional status of elderly patients, and the role of microtransplantation compared with traditional transplantation 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 possibly for a minimal residual disease–stratified high-risk group in patients with favorable cytogenetic risk category. Elderly patients with good performance status (Eastern Cooperative Oncology Group performance status 0 to 1) may need more aggressive treatment than chemotherapy alone and future studies will determine the best postremission therapy in these patients.

Dr. Ephraim Fuchs discussed advances in HaploSCT for patients with hemoglobinopathies, especially sickle cell disease (SCD). In this disease, engraftment is now possible in patients with a major HLA–mismatched 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 (>10 g/dL). Other diseases with a high graft failure rate after transplantation with the current RIC regimen (Flu/Cy/TBI) were myeloproliferative neoplasm and chronic lymphoblastic leukemia, in the range of 40% to 45%. Because of the fairly high graft failure rates, 3 days of ATG were added at Johns Hopkins to the backbone regimen of the Flu/Cy/TBI regimen, with a later change of tacrolimus to sirolimus and priming the bone marrow donor with granulocyte colony–stimulating factor (G-CSF), which improved the harvest cell count from 4.69×10^8/kg to median of 12×10^8/kg. Without adding G-CSF after day 5, engraftment of neutrophils approached 90% after a median of 25 days, allowing a cure rate of approximately 50% with no TRM (unpublished data). Outcomes varied when different related donors were used; they were best with a child (100% engraftment) or brother (75%) as donor. The disadvantage to adding G-CSF priming was an increase in the rate of grades II to IV aGVHD to approximately 50%, 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 cGy or 400 cGy, which was not tested yet. Sirolimus-induced tolerance could not be achieved at least in the 3 patients of that cohort. When stopping sirolimus was attempted, previously stable mixed chimerism began to deteriorate; however, chimerism was restored after restarting the drug. Dr. Fuchs also discussed the National Institutes of Health protocol of allogeneic hematopoietic stem cell transplantation (HSCT) for patients with SCD using an alemtuzumab/TBI-based preparative regimen and PTCy with sirolimus-based GVHD prophylaxis. Engraftment was excellent (100%, n = 7) after using 2 days of Cy (100 mg/m^2) and success rates were reported to be approximately 70% (n = 5 of 7). aGVHD 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%.

Ongoing and Future Clinical Trials in Haploidentical Transplantation

Dr. Ephraim Fuchs discussed the updates of active and planned Blood and Marrow Transplant Clinical Trials Network (BMTCTN) studies involving haploidentical transplantations. BMTCTN 1101 is a phase III randomized trial of nonmyeloablative conditioning randomizing patients to either double umbilical cord transplantation (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 transplantations. The trial opened in mid-2012 with 35 centers

activated and 6 more to be activated in the near future. In addition, the German cooperative group (DKMS) 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. Allowing the use of PBSC grafts for haploidentical transplantations may improve accrual to this study.

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. 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 enrollment over 2 years will be 31 patients. Results of this study will be compared with those of MUD transplantations reported to CIBMTR. Dr. Fuchs presented the proposed new studies to BMTCTN, including CBT versus HaploSCT for aplastic anemia. The primary hypothesis in this study 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 transplantation group will be with PTCy, MMF, and tacrolimus with bone marrow (preferred) or PBSC for graft source. This study was postponed; however, 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 incidences of aGVHD and cGVHD were very low and no patient died from these complications. The absence of cGVHD has to be valorized in view of the long life-expectancy of pediatric patients. Patients given this type of allograft for a nonmalignant disorder have a probability of disease-free survival (DFS) of roughly 90%, although some patients experienced graft failure and few others died from viral complications [25]. This program offered the Rome group an 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 CMV reactivation and are more cytotoxic than 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 $\gamma\delta$ T cells. Although recovery of innate immunity was prompt with this approach of HaploSCT, recovery of adaptive immunity is still suboptimal 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 started, in November 2014, a phase I/II study enrolling children with either malignant or nonmalignant disorders who will receive TCR-$\alpha\beta$/B cell–depleted HaploSCT, followed by the infusion of titrated numbers of iC9 T cells on day 14 ± 4. These iC9-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 aGVHD occurs.

With concern regarding the higher incidence of aGVHD and possible high risks associated with this procedure, transplantation 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 who underwent transplantation from a matched donor, either an MRD or an MUD. All 3 groups were comparable except for conditioning (70% of patients with a matched donor received nonmyeloablative conditioning whereas 100% of HaploSCT patients received a RIC, which consisted of Flu [5 days], busulfan [2 days], and ATG [2 days]), and GVHD prophylaxis (HaploSCT patients received PTCy with MMF/cyclosporine whereas matched transplant recipients received conventional GVHD prophylaxis). All patients engrafted but 1 in the HaploSCT group (97%, n = 30 of 31). Incidences of grades II to IV aGVHD were not statistically different between haploidentical and MRD transplantations; however, it was significantly lower when compared with the rates for MUD group. Severe cGVHD was reported to be significantly lower in HaploSCT group than in those who received MRD and MUD grafts (0% versus 16% versus 14%, $P = .02$ and .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 overall survival and progression-free survival were significantly better after haploidentical than after MUD transplantation (70% versus 51% and 67 versus 38%, $P = .08$ and $P = .02$, respectively) but did not statistically differ from MRD transplantations. Dr. Blaise concluded that T cell–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, and haploidentical donors. Conditioning was busulfan-based in myeloid malignancies and TBI-based in lymphoid malignancies. The source of the graft was PBSC for the great majority of patients. MMF and tacrolimus were given until days 35 and 100, respectively. 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 transplantations, respectively. Correlative studies on immune reconstitution revealed that recovery of regulatory T cells (Tregs) was relatively fast, but $\gamma\delta$ T cell

reconstitution was very slow, especially in patients who received a transplant from haploidentical donors. γδ T-cells are not HLA restricted (ie, they do not cause GVHD) but attack infected cells or with neoplastic transformation, and because γδ T-cells have been shown to improve survival after haploidentical stem cell transplantations by decreasing the relapse rate without increasing the incidence of aGVHD in 153 patients with acute leukemia [20], a clinical trial in haploidentical stem cell transplantations 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 antitumor 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% to 11.9% of $CD3^+$ cells (before ABD) to above 97% (after ABD). Using apheresis, 4×10^6 CD34 cell/kg will be collected and infused on transplantation day 0. The remaining product will be ABD 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 obtained from 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 antigen-presenting cells expressing mbIL-21 to decrease relapse rate after transplantation for patients with myeloid malignancies (AML, MDS, chronic myeloid leukemia). There are several reasons to administer NK cells after transplantation: NK cells generated early after transplantation both in HLA-matched and T cell–depleted haploidentical transplantation are functionally immature with low KIR expression, 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 to 60 day post-transplantation period has been associated with a decrease relapse rate and increased survival [33,34]. In haploidentical transplantation with PTCy, the MD Anderson group showed that patients had the lowest NK cell numbers and lowest function on day 30 after transplantation. 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 after transplantation. In addition, higher doses obtained from ex vivo expansion would enhance the antitumor effects of the graft. NK cells ware infused on days -2, +7, and on/after +28 after transplantation. The first infusion was with fresh and the other 2 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, it was evaluated in all patients. Three patients were treated to date, 1 at 1×10^4/kg dose and 2 at 1×10^5/kg. Infusions were not associated with toxicities or the development of aGVHD.

Cellular Therapy and Immune Reconstitution

Dr. Massimo Martelli presented updated data regarding using Tregs infused with conventional T cells (Tcons) after "mega-dose" $CD34^+$-selected HaploSCT in 52 patients with high-risk acute leukemia. Patients with AML (20 CR1, 17 ≥ CR2) and ALL (10 CR1, 5 in ≥ CR2), median age of 39 years, were treated between September 2008 and February 2014. All patients who underwent transplantation 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 Flu (40 mg/m^2/day from day -10 to -6). Forty-eight percent (n = 25 of 52) of patients received 35 mg/kg Cy from days -8 and -7 and 44% (n = 23 of 52) patients were given alemtuzumab or thymoglobulin instead 21 days before transplantation to prevent interference with Treg-Tcons adoptive immunotherapy. Finally, under the latest protocol 8% (n = 4 of 52) received a reduced dose of Cy (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 after transplantation. Sustained full donor engraftment was achieved in the majority of patients (96%, n = 50 of 52). Even though 1.1×10^6/kg ± .6 Tcons had been infused, only 12% (n = 6 of 50) of evaluable patients developed ≥ grade II aGVHD and 2% (n = 1 of 52) patient developed cGVHD. There was a rapid, sustained increase in peripheral blood T cell subpopulation recovery. $CD4^+$ and $CD8^+$ counts reached 100/μL at a median of days 40 (range, 25 to 150) and 45 (range, 18 to 100) after transplantation, respectively. Compared with T cell–depleted HaploSCT, $CD4^+$ and $CD8^+$ specific for opportunistic pathogens such as *Aspergillus fumigatus*, *Candida albicans*, CMV, adenovirus, herpes simplex virus, varicella zoster virus, and toxoplasmosis emerged significantly earlier (at each time point $P < .0001$).

Overall, at a median follow-up of 4 years (range, 7 to 58 months) the cumulative incidence of TRM was 40% and DFS was 58% (n = 30 of 52). In patients receiving anti-T cell antibodies or lower dose of Cy, TRM was 23% and DFS was 70%. Only 5% (n = 2 of 41) evaluable patients relapsed. These patients had evidence of minimal residual disease at the time of transplantation 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, .06; 95% CI, .02 to .35; $P = .02$).

Murine mouse models infused with human primary AML cells alone or with Tcons died of leukemia and GVHD, respectively. Coinfusion of 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, whereas Tcons that home to the bone marrow exerted unopposed alloantigen recognition. This phenomenon could be related to the Treg migratory properties, as $CD45RO^+$ 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 cotransplantation of enough Tcons to eradicate minimal residual disease, thus

eliminating the usual 30% to 35% incidence of post-transplantation high-risk acute leukemia 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 (GVL) 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. The degree of sensitivity to NK-mediated killing is often correlated to 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 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–depleted haploidentical transplantation [37]. Further work showed that donors with KIR B haplotypes (typically containing 1 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 transplantation, 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 IFNγ 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–depleted and T cell–replete HSCT, with further recovery of killing at 6 months. By contrast, at 3 months after T cell–replete 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, 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. Before NK cell infusion, patients underwent a preparative regimen that involved 3 different chemotherapy preparations: high-dose Cy and Flu, low-dose Cy and methylprednisone or Flu. After infusion, patients received IL-2 daily for 14 days. NK cell expansion was only observed for patients receiving the preparatory regimen of high-dose Cy and Flu. Successful expansion of NK cells was determined by the detection of greater than 100 NK cells/uL of blood 12 to 14 days after infusion. On this protocol, 30% of poor-prognosis AML patients achieved CR. Higher rates can be achieved when combined with IL-2 diphtheria toxin fusions intended to deplete Tregs [40]. Long-term DFS was achieved only when this was followed by allogeneic transplantation.

Although 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 transpresentation [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 IL-15, produced by Steven Creekmore's group at the National Cancer Institute, in its unbound form (monomeric) was used to in vivo expand adoptively transferred NK cells. A first in human trial treating post-transplantation relapse using IL-15/IL-15Rα-Fc super agonist complexes is ongoing, the design of which includes a mutant IL-15, the addition of a sushi domain to inhibit complement activation, increase avidity of the molecule to IL-2R βγ on NK cells, and increase 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 CMV infection [48,49]. In humans, $CD57^+NKG2C^+$ NK cells specifically expand in response to human CMV [50-52] and are 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 (antibody-dependent cell-mediated cytotoxicity) 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 believed that these cells are optimally primed by CMV to change the repertoire of NK cells to fight cancer.

Another direction is to develop strategies to bind NK cells to target antigens by developing bi- and trispecific killer engagers (fusions of the scFv (single-chain variable fragment) of anti-CD16 with 1 or more target Ag) [55,56]. It has been shown that anti-CD16x33 bispecific killer engager activation overcomes inhibitory signaling via class I HLA to potently kill primary cancer targets [57] as well as targeting $CD33^+$ myeloid-derived suppressor cells [58].

Dr. Carl June discussed strategies for consolidation therapies after allogeneic transplantation using genetically engineered T cells. The rationale that supports this approach is that there is an emerging long-term safety profile after 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 human immunodeficiency virus and various forms of hematologic malignancies [59]. Importantly, there have been no incidences of transformation or genotoxicity after 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 GVHD than would be expected after DLI [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 allogeneic HSCT and prior DLI from the original donor [63]. In this phase I study, 10 patients were given infusions of CD19 CAR T cells manufactured from each patient's original HSCT donor; in 6 cases from a matched sibling donor and in 4 cases, the donor was unrelated. There were no significant cases of GVHD. There was some evidence of antitumor activity with 1 patient with refractory chronic lymphoblastic leukemia achieving a CR and 1 partial remission 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, at a median age of 11 years (range, 4 to 24), have been infused with a median of 4.3×10^6 CTL019 cells/kg (range, 1 to 17.4×10^6/kg). In 35 of the 53 patients, the T cells were collected from patients who had relapsed after allogeneic HSCT and in all cases were of donor origin in 100% donor. No cases of aGVHD or cGVHD were observed. Whether autologous or allogeneic CAR T cells were infused, the response rate did not differ, as 50 patients (94%) achieved 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 toxicity if 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 third-party donors could be feasible and have antileukemic efficacy.

Dr. Hui-Sheng Ai discussed the concept and clinical investigation of microtransplantation, which consists of utilizing a conditioning regimen that 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 Flu) to preserve recipient immune function. This is followed by programmed infusion of G-CSF–mobilized allogeneic PBSC (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; GVL and recipient-versus-leukemia effects are induced with avoidance of clinical aGVHD [65]. Updated data on microtransplantation for treating elderly AML patients were reported. One hundred three AML patients ages 60 to 88 years were assigned to receive induction chemotherapy with mitoxantrone and cytarabine with ($n = 75$) or without ($n = 28$) G-CSF–mobilized HLA-mismatched PBSC (G-PBSCs) infusion. Patients who achieved CR received 2 other cycles of consolidation with intermediate dose cytarabine with or without G-PBSCs. The CR 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 versus 16 days) and platelet count recovery was (15.5 versus 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 multicenter 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 3 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 ($\geq 1.1 \times 10^8$/kg) with each infusion had a higher 6-year leukemia-free survival (76.4% versus 49.5%) and overall survival (82.1% versus 55.3%) than those receiving a lower dose ($< 1.1 \times 10^8$/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 accelerated hematopoietic recovery and improved CR rates and survival. This appeared to separate GVL from GVHD and was done across a major HLA barrier. An open, randomized, controlled, and international multicenter 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 also briefly reviewed data suggesting favorable immune reconstitution marked by low incidence of invasive viral infections and Epstein-Barr virus–related post-transplantation lymphoproliferative disease when PTCy was used to prevent GVHD [68]. Dr. Luznik acknowledged that the in vivo mechanisms of PTCy and in particular, the immune dynamics occurring during the first year after allogeneic HSCT using PTCy, remain poorly understood and that there are 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 to 2 months after PTCy is characterized by persistence of activated 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, the enzyme primarily responsible for in vivo detoxification of Cy, upon allogeneic stimulation in a lymphopenic environment [70,71]. Finally, Dr. Luznik presented data on the assessment of immune reconstitution in 71 patients undergoing myeloablative conditioned HaploSCT with PTCy (50 mg/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 myeloablative conditioned HLA-matched allogeneic HSCT with PTCy (50 mg/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 to 3 months. By 1 year after HaploSCT and HLA-matched HSCT, median absolute lymphocyte counts were in the normal range (1100 to 4800 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 ($P < .0001$). Median $CD4^+$ T cell counts at 1 year were significantly lower after both

HLA-matched and HaploSCT than they were for those who underwent transplantation with normal donors. At 6 months and 1 year after transplantation, there was no significant difference in $CD8^+$ T cells after HaploSCT or HLA-matched HSCT compared with that for with normal donors. There was a trend towards lower total $CD8^+$ T cell counts at all time points after HaploSCT compared with after HLA-matched HSCT. Phenotypic effector memory and terminally differentiated effector memory T cells 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, there is comparable reconstitution of NK and B cells; however, early recovery of $CD4^+$ and $CD8^+$ T cell lags after HaploSCT when compared to recovery after 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 will help further decipher the T cell repertoire reconstitution after HSCT with PTCy, particularly the effect of clinical variables on its diversity.

Dr. Enrico Lugli discussed cellular mechanisms of T cell reconstitution after haploidentical transplantation early after T cell–replete transplantations 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 after recognition of alloantigen, although evidence in humans remains elusive. The optimal scenario in this setting would be to spare nonalloreactive 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 tracking of T cell dynamics during the early days and weeks after T cell–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-transplantation 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–specific T cells are mostly TN in healthy donors: the acquisition of memory/effector phenotypes by these cells in the recipient would suggest they progressed through an early TSCM stage in the post-transplantation environment. CD8 + T cells specific for MART1 and Wilm's tumor-1 epitopes could be detected in patients up to 90 days after 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 $CMV^{+/-}$ transplantations) 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/tumor-associated antigen–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-transplantation infectious complications.

Dr. Marcel van den Brink discussed strategies to enhance post-transplantation T cell reconstitution. T cell deficiency after allogeneic transplantation remains an issue 1 to 2 years after transplantation, which increases 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 is faster in younger patients than in older patients; this is probably the result of thymic dysfunction in aging population. Other factors associated with thymic dysfunction include conditioning (ie, chemotherapy, radiation, and the use of antibodies) and GVHD. Several strategies to enhance T cell recovery after allogeneic HSCT, which are currently in trials or in development, were discussed, including interleukin-7, keratinocyte growth factor, sex steroid inhibition, and administration of T cell precursors.

Some of the nonhematopoietic stromal cells that support thymopoiesis include fibroblast, epithelial, and endothelial cells. This support is done though important thymopoietic factors involved in T cell commitment, such as Notch ligand DLL1 and DLL4, migration, such as CCL21, CXCL12, and CCL25, as well as proliferation cytokines stem cell factor, IL-7, and IL-15. The thymus is exquisitely sensitive to negative stimuli (ie, 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, including delta-like ligand 4. Targeting luteinizing hormone–releasing hormone receptor promotes thymic regrowth through enhancement of delta-like ligand 4 expression, subsequently enhancing immune recovery after acute thymic damage and peripheral T cell function. This was seen in both genders.

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, promote an increased number of both thymocytes and TECs, and enhance T cell development during GVHD through increased thymopoiesis and exporting of more naïve T cells 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 transplantations have improved, now approaching outcomes of HLA-matched transplantations. Haploidentical transplantations are an area of active investigation in transplantation with novel approaches focused on better controlling of alloreactive reactions, elimination of post-transplantation immunosuppression, prevention of disease relapse, and improvements in immunologic reconstitution. Multiple exciting clinical trials that are ongoing will likely further advance our knowledge and improve outcomes of patients treated with haploidentical donors over the next several years.

ACKNOWLEDGMENTS

The organizers thank Bellicum Pharmaceuticals, Kiadis Pharma, and Miltenyi Biotec for their support in organizing this meeting.

Financial disclosure statement: The authors have no financial disclosures.

Conflict of interest statement: D.C.R. has received research support from Kiadis Pharma. Other authors have no conflict of interest to declare.

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