

## REVIEW

# *Ex vivo* T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future

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The most common cause of post-transplant mortality in patients with hematological malignancy is relapse, followed by GvHD, infections, organ toxicity and second malignancy. Immune-mediated complications such as GvHD continue to be challenging, yet amenable to control through manipulation of the T-cell compartment of the donor graft with subsequent immunomodulation after transplant. However, risk of both relapse and infection increase concomitantly with T-cell depletion (TCD) strategies that impair immune recovery. In this review, we discuss the clinical outcome of current and emerging strategies of TCD in allogeneic hematopoietic stem cell transplant that have developed during the modern transplantation era, focusing specifically on *ex vivo* strategies that target selected T-cell subsets.

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## INTRODUCTION

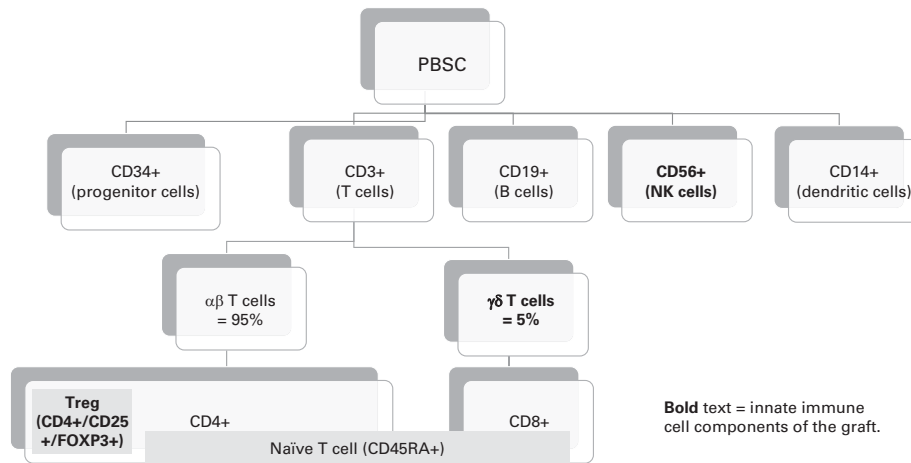
High-dose chemo/radiotherapy followed by allogeneic hematopoietic stem cell transplant (HSCT) provides a potentially curative treatment for a variety of hematological diseases. The commonest cause of post-transplant mortality is relapse of neoplastic disease (41%), GvHD (12%), infections (11%) and organ toxicity.<sup>1</sup> Although attenuated conditioning regimens can decrease the risk of organ toxicity, alloreactive lymphocytes of the graft can mediate a potentially life-threatening GvHD due to HLA dissimilarity.<sup>2,3</sup> Moreover, the majority of patients (~70%) do not have matched sibling donor<sup>4</sup> and thus require alternative donors that could have greater degrees of HLA disparity, increasing the risk of GvHD. Indeed, the initial attempts using unmanipulated marrow from alternative donors resulted in severe GvHD.<sup>5,6</sup> Preclinical models showed that both CD4+ and CD8+ T cells are capable of mediating lethal GvHD in HLA-incompatible transplants.<sup>7</sup> The recognition of the graft versus tumor (GVT)<sup>8</sup> phenomenon after bone marrow (BM) transplantation likely contributed to the increasing use of PBSC grafts in order to exploit the anti-neoplastic function of the cytotoxic T cells in the PBSC graft (PBSC grafts have one log more T cells than BM grafts). PBSC graft is conceivably easier to collect and has been associated with faster engraftment.<sup>9</sup> However, the use of PBSC has contributed to an increased risk of GvHD, in particular chronic GvHD. This has been shown in the setting of matched sibling<sup>10</sup> and matched unrelated donors.<sup>9</sup> Thus, the concept of separation of GvHD and GVT was coined and captured the attention of several investigators.<sup>11</sup>

## METHODS OF GRAFT MANIPULATION

T cells are major component of the hematopoietic stem cell graft (Figure 1) exerting an adaptive or innate immune response (Table 1). Graft manipulation is commonly done via 'depletion' of T cells that are implicated in GvHD or less commonly 'expansion'

of regulatory T cells (Treg: CD3+ CD4+ CD25hi FoxP3+) to reduce GvHD risk, or NK and  $\gamma\delta$  T cells to decrease risk of relapse and enhance immune reconstitution (Table 2). Various methods have been employed for TCD (Table 3). Initial attempts to remove the T cells from the hematopoietic graft *ex vivo* were attempted in the late 1980s<sup>12</sup> via agglutination with soybean lectin and rosetting the residual T cells with sheep RBC, and this was further advanced to the use of T-cell-directed monoclonal antibodies, for example, anti-CD2, CD3, CD5 in combination with panning, immunotoxin, or complement (to enhance elimination of antibody-sensitized cells).<sup>12–14</sup> These trials using pan-TCD showed initially promising results by marked reduction of risk of GvHD even without the use of post-transplant pharmacological GvHD prophylaxis. However, this was associated with an increased risk of disease relapse seen particularly in patients with CML.<sup>15</sup> In addition, an increased incidence of graft failure was observed, in both matched related donors,<sup>16</sup> and alternative donors,<sup>17</sup> suggesting that donor T cells are required to counter balance the ability of residual recipient T cells (surviving conditioning regimen) to reject the graft. These findings strongly suggested the same alloreactive T cells responsible for GvHD could also be beneficial in both facilitating engraftment and eliminating residual leukemia through an adoptive immune response of the GVT effect.<sup>8</sup> Thus aggressive *ex vivo* pan-TCD seemed not to be optimal even for alternative donor transplants, and subsequent studies have explored the use of modified or targeted TCD that leaves more T cells in the graft combined with post-transplant pharmacological immunosuppression.

Alternative to *ex vivo* T-cell depletion, serotherapy has been used for *in vivo* T-cell depletion. This has been done using either as anti-thymocyte globulin (ATG),<sup>18</sup> or alemtuzumab.<sup>19</sup> While alemtuzumab use has declined due to increased risk of relapse and engraftment failure in particular with haploidentical (haplo) HSCT, ATG continues to be more frequently used at variable doses. A CIBMTR retrospective analysis showed lower risk of acute and



**Figure 1.** Major components of apheresis and bone marrow grafts with predominately innate lymphocyte components highlighted in bold. A full color version of this figure is available at the *Bone Marrow Transplant* journal online.

	<i>Clinical significance</i>
Adaptive immune system (antigen-specific) T cells → cell-mediated (cellular) immunity. B cells → antibody-mediated (humeral) immunity.	Fight infection Kill neoplastic cells (GVT effect) Cause GvHD
Innate immune system (nonspecific) <sup>a</sup> NK cells. <sup>b</sup> Treg γδ T cells.	Fight infection Kill neoplastic cells (GVT effect) Does not cause GvHD

Abbreviations: GVT=graft versus tumor; NK=natural killer. <sup>a</sup>Also includes granulocytes (neutrophils, eosinophils and basophils), monocytes and macrophages. <sup>b</sup>NK cells can have adaptive and innate immune functions.

<i>Manipulation strategy</i>	<i>Clinical purpose</i>
Depletion (targeted negative selection) Pan T-cell depletion <i>Ex vivo</i> (with or without T-cell add-back) <i>In vivo</i> serotherapy (ATG or alemtuzumab). T-cell subset depletion CD8+ T-cell depletion. CD3/CD19 cell depletion. αβ T-cell/CD19 cell depletion. Naive T-cell depletion.	GvHD risk reduction
Depletion of immune cells (indirectly) CD34+ positive selection.	
Inclusion (positive selection/expansion) Treg cells	GvHD risk reduction
NK cell γδ T cells	Relapse/infection risk reduction

Abbreviations: ATG = anti-thymocyte globulin; NK = natural killer.

chronic GvHD and higher risk of relapse with either method of serotherapy compared with T-cell replete transplant (PBSC or BM).<sup>20</sup> Another evolving method of *in vivo* alloreactive T-cell depletion is use of post-transplant cyclophosphamide (PTCy). This method has been clinically introduced with T-cell replete haplo BM transplant<sup>21</sup> and is becoming increasingly used with PBSC graft<sup>22</sup> as well as with HLA-matched<sup>23</sup> transplant. Detailed

discussion of *in vivo* T-cell depletion is beyond the scope of this article.

**T-CELL DEPLETION AND CD34+ CELL SELECTION IN HLA-MATCHED HSCT**

The initial trials of *ex vivo* TCD using monoclonal antibodies was associated with high risk of GvHD.<sup>24</sup> Investigators soon realized that additional treatment of the T cells with complement or immunotoxins (along with anti-T-cell antibody) is essential to eliminate the T cells from the graft. This approach resulted in ~2–3 log reduction of the T cells and was associated with lower risk of GvHD of 10–20% without pharmacological GvHD prophylaxis.<sup>25</sup> Graft failure was also a hindrance to initial clinical studies using *ex vivo* T-cell depletion of BM graft even with HLA-matched donors.<sup>16,26</sup> Subsequent data from Memorial Sloan-Kettering Cancer Center (MSKCC) showed encouraging results with low risk of GVHD and no increased risk of relapse after myeloablative BM transplant.<sup>27,28</sup> A National Marrow Donor Program analysis of patients who underwent BM transplant from HLA-matched unrelated donor between 1987 and 1990 included data on 95 patients who underwent TCD.<sup>29</sup> This showed TCD was associated with less risk of acute and chronic GvHD, higher risk of secondary graft failure and comparable survival outcome to T-cell replete marrow transplant. The Center for International Blood and Marrow Transplant Research (CIBMTR) also analyzed data of 2254 patients who underwent HLA-matched transplant with or without T-cell depletion.<sup>8</sup> In this seminal analysis, the

**Table 3.** Methods of T-cell depletion

<i>Ex vivo</i> methods	<i>In vivo</i> methods
Negative selection of T cells	Polyclonal ATG
Soybean lectin agglutination with E-rosette depletion.	Atgam (horse)
Antibody-mediated	Thymoglobulin (rabbit)
Monoclonal antibody with complement or immunotoxin.	ATG-Fresenius (rabbit)
Monoclonal antibody with immunomagnetic beads.	Monoclonal antibody
Positive selection of CD34+ cells	Alemtuzumab (anti-CD52).
Monoclonal antibody with immunomagnetic beads)	

Abbreviation: ATG = anti-thymocyte globulin.

**Table 4.** Outcome of studies utilizing *ex vivo* T-cell depletion or CD34+ selection in HLA-matched HSCT

Year published	n	Graft type	Study design	aGvHD (grades II–IV)	cGvHD	Relapse	Other findings
1992 <sup>90</sup>	31	BM	Phase II	10% (any grade)	None (median FU 72 months).	13%	Graft failure: 16% DFS at 3 years: 45%
1998 <sup>91</sup>	31	BM	Phase II	None	6%	6%	ATG used in addition to <i>ex vivo</i> T-cell depletion No graft failure DFS at 4 years (AML in CR1 under age of 40 years): 81% DFS at 3 years: 27% and 34% (P = 0.16)
2005 <sup>68</sup>	405	BM	Randomized multicenter phase III (TCD with CSA versus CSA/MTX)	39 versus 63% (P < 0.0001)	No difference.	Relapse of CML: 20 versus 7% (P = 0.009)	CMV infection: 28 Versus 17% (P = 0.023)
2008 <sup>27</sup>	49	PBSC/BM	Retrospective	6% (any aGvHD)	2%		Graft failure: 9% OS at 3 years: 37% NRM at 2 years: 30%
2011 <sup>32</sup>	44	PBSC	Phase II (multicenter)	23%	19% (2 years) 7% (extensive cGvHD)	24%.	All patients engrafted DFS at 3 years: 53%
2011 <sup>92</sup>	35	PBSC	Phase II	9%	29%	6%	Secondary graft failure: 1 patient. DFS at 4 years: 57% Infection-related mortality: 14%
2015 <sup>28</sup>	102	PBSC	Retrospective	16%	4% (1 year)	16%	Graft failure: 4% OS at 5 years: 49% DFS at 5 years: 48% NRM at 5 years: 33%

Abbreviations: BM = Bone marrow; CSA = cyclosporine; FU = follow up; MTX = methotrexate; PBSC = peripheral blood stem cell.

phenomenon of graft versus leukemia effect was emphasized by showing higher relapse rate among TCD group.

Investigators also explored the use of CD34+ cell selection rather than TCD in order to enhance the elimination of other lymphocyte or immune cell components in the graft that could be implicated in the pathogenesis of GvHD.<sup>30</sup> This interest became notable with the emergence of PBSC grafts as an alternative to BM grafts, in particular when the former was shown to be associated with increased risk of chronic GvHD.<sup>9,31</sup> Myeloablative regimen has been preferred with TCD to mitigate risk of engraftment failure. In 2005, the Blood and Marrow Transplantation Clinical Trials Network (BMT-CTN) initiated a multicenter phase II trial of myeloablative PBSC transplant on AML patients in complete remission (n = 44) (BMT-CTN 0303) with CD34+ cell selection and no other pharmacological GvHD prophylaxis.<sup>32</sup> The study showed successful engraftment of all accrued patients with low incidence of acute and chronic GVHD. The result of this study (BMT-CTN 0303) was compared with the cohort of BMT-CTN 0101 trial that involved T-cell replete HSCT (with calcineurin inhibitor (CNI)-based GvHD prophylaxis regimen).<sup>33</sup> This showed no statistical difference in the rate of grades II–IV aGvHD. However, the rate of cGvHD (at 2 years) was significantly lower in the

TCD patients (19 versus 50%, P < 0.001). There were no differences in the risk of engraftment failure, relapse, non-relapse mortality (NRM), disease-free survival (DFS), or overall survival (OS). Two studies have also compared the outcome of TCD versus T-cell replete HSCT (retrospective comparison between data from MSKCC and the MD Anderson Cancer Center) showed lower risk of acute and chronic GvHD in the TCD group with no difference in DSF and OS.<sup>34,35</sup> It is to be noted that discrepancies in the outcome of these studies (Table 4) may be attributed to difference in the efficiency (log depletion) of T cells, HLA disparity (low resolution testing was only used in the earlier studies), and different patient population and conditioning regimens. With the increasing use of PTCy, BMT-CTN 1301 study (NCT02345850) was initiated randomizing HLA-matched transplant (sibling or unrelated) into 3 arms; CD34+ selection, CNI-based regimen, PTCy. Results of this trial would be pivotal in comparing *ex vivo* TCD and alloreactive TCD utilizing PTCy.

#### CD8+ T-CELL DEPLETION

Since the initial results of *ex vivo* TCD remained suboptimal with risk of poor or delayed immune reconstitution and engraftment

**Table 5.** Studies of *ex vivo* CD8+ cell depletion in HLA-matched HSCT

Year published	n	Graft type	Study design	aGvHD (grades II–IV)	Relapse	Other findings
1990 <sup>93</sup>	36	BM	Phase II	28%	11% (none of the 13 cases with CML relapsed)	Graft failure: 8% OS at 2 years: 57%
1994 <sup>94</sup>	38	BM	Randomized double-blinded trial of CD8+ depletion versus none)	20% (CD8+ depletion) versus 80%	10% in both arms	Graft failure: 10% (CD8+ depletion) versus none DFS at 3 years similar (37%)
1998 <sup>95</sup>	40	DLI (salvage)	Phase II	15%	N/A	Response: 80% (in CML and MM)
2002 <sup>96</sup>	18	DLI (prophylactic)	Randomized trial of CD8+ depleted DLI versus none) given 6 months post-transplant	None (CD8+ depleted) versus 56% in control arm	11% in CD8+ depleted arm versus 33% in control arm	
2004 <sup>97</sup>	9	DLI (salvage)	Phase II	10%	N/A	Response: 44% (including all 3 patients with CML)
2004 <sup>36</sup>	41	PBSC	Phase II	61%	5%	Engraftment: 100% cGvHD: 50% EFS at 2 years: 57%

Abbreviations: BM = bone marrow; DLI = donor lymphocyte infusion; EFS = event-free survival; PBSC = peripheral blood stem cell.

failure, attempts were made to selectively deplete CD8+ T cells as the effector cytotoxic cells that mediate GvHD tissue injury. However, CD8+ cell depletion studies (Table 5) failed to mitigate the risk of GvHD as evidenced by risk of acute GvHD grades II–IV 61% in a phase II study of HLA-matched PBSC transplantation.<sup>36</sup> These data suggested that the distinction between GvHD and GVT is not a simple dichotomy of T-cell subsets (CD4+ and CD8+).<sup>36,37</sup> The CD8+ cell depletion also likely depleted CD8+ NK cells (~20–30% of total NK cells) and CD8+  $\leftrightarrow$   $\gamma\delta$  T cells (20%).

### T-CELL DEPLETION WITH T-CELL ADD-BACK

The earliest attempt to exploit the concept of adoptive immunotherapy by infusion of add-back immune cells was done by the Fred Hutchinson Cancer Research Center group when they performed a study testing the impact of infusion of add-back ‘bone marrow buffy coat’ after ‘unmanipulated’ BM transplantation (BMT).<sup>38</sup> The purpose of this approach was to explore the potential anti-leukemic effect of the add-back of immune cells to decrease risk of relapse after BMT. However, increased risk of GvHD hindered further progress of this approach. Interest in this strategy was revived in the era of TCD. Clinical trials using add-back donor T-cell infusion following *ex vivo* TCD HSCT was done at the National Institute of Health, USA.<sup>39–42</sup> The most updated results from this group reported data of 138 patients with hematological malignancies who received myeloablative TCD PBSC transplant from an HLA- identical sibling donor.<sup>42</sup> In this study, one or two add-back products were infused as  $1 \times 10^7$  T-cells per kg between day +45 and/or day +100. With a median follow up of 4 years, the OS and DFS, relapse and NRM were 58%, 46%, 40% and 20%, respectively. The incidence of grades II–IV aGvHD and cGvHD were 39% and 36% respectively. In another prospective study, children with HLA-matched or mismatched unrelated PBSC transplant ( $n=19$ ) received T-cell add-back following TCD transplant.<sup>43</sup> CN1 alone was used for GvHD prophylaxis and the risk of grades II–IV aGvHD and extensive cGvHD was 16 and 0%. All patients engrafted and NRM at 1 year was 6% with 1-year OS of 82%. More recently, suicide gene (caspase-9) programming of add- back T cells has enabled elimination of T cells (upon treatment with suicide gene-activating drug) upon development of severe GVHD.<sup>44</sup> Results of this approach used in 10 pediatric patients shows favorable protection against viral infection after a 16-month follow up.<sup>45</sup>

This approach continued to be evaluated in an ongoing clinical trial (NCT01744223). Another clinical trial (NCT02500550) is evaluating the effect of photodynamic depletion/inactivation of alloreactive T cells in add-back infusion.<sup>46</sup>

### T-CELL DEPLETION AND CD34+ CELL SELECTION IN HAPLO HSCT

One of the earliest studies of *T-cell replete* haplo BMT ( $n=35$ ) reported by The Royal Marsden Hospital (UK) revealed a very high risk of graft failure and GVHD.<sup>47</sup> Subsequent similar studies by the Fred Hutchinson Cancer Research Center group showed that mismatching at two out of six loci or more has the same detrimental consequences.<sup>2,5</sup> Initial attempts at TCD depletion were concurrently being tested to overcome the HLA disparity barrier with a 1981 report from MSKCC showing successful haplo BMT in an infant with AML after *ex vivo* TCD using differential agglutination with soybean agglutinin and sheep RBC rosette depletion.<sup>48</sup> No GVHD occurred after this transplant. Subsequently, a separate study reported successful engraftment of two out of three patients with SCID receiving haplo donor BM grafts.<sup>49</sup> Using the same method (soybean agglutination and E-rosetting), Perugia University (Italy) introduced the use of the ‘CD34+ mega dose’ concept in the early 1990s in an effort to overcome the risk of engraftment failure encountered with TCD. G-CSF mobilized stem cells were T-cell depleted and added to the BM graft to enrich the CD34+ stem cell dose (mega dose approach).<sup>48</sup> With the advent of monoclonal antibodies (mAbs) against the TCR in early 1990s, several studies combined either TCD or CD34+ selection methods with systemic ATG and pharmacological GVHD prophylaxis (CNI with or without corticosteroid) showing improved results over previous attempts.<sup>50–55</sup> With these approaches grades II–IV aGvHD was reduced to 13 to 40%, engraftment failure ~ 10% and OS at or below 50% (Table 6). Recently, the use of high-dose post-transplant cyclophosphamide following infusion of a T-cell replete graft is revolutionizing haplo HSCT.<sup>21,22</sup> However, there remain several unmet needs to improve haplo HSCT outcome such as improving post-transplant immune reconstitution, which may also decrease relapse rate (in particular with the use of reduced intensity conditioning regimens).

**Table 6.** Outcome of studies utilizing *ex vivo* T-cell depletion or CD34+ positive selection in haplo HSCT

Year published	n	Graft type	Study design	GVHD (grades II–IV)	cGVHD	Relapse	Other findings
1994 <sup>48</sup>	17	BM+ PBSC (mega dose)	Phase II	1/17 (received higher T-cell dose than all others)	Not reported	12%	Engraftment failure: 1/17 NRM: 53%
1996 <sup>50</sup>	40	BM	Phase II	36%	17% (extensive)	11% (at 2 years).	Engraftment: 93% (similar to historical control of n = 17, P = 0.12) OS at 5 years: 40%
1997 <sup>53</sup>	27	BM	Phase II	40%	19%	11%	Engraftment: 89% OS at 2 years: 56%
1997 <sup>52</sup>	72	BM	Phase II	16%	35% (extensive in 8%)	32%	Engraftment: 88% OS at 2 years: 55 and 27% in low- and high-risk groups (P = 0.05)
1998 <sup>54</sup>	43	PBSC (mega dose) +BM (only n = 28)	Phase II	0%	0%	30%	Engraftment: 100% NRM: 40%
2004 <sup>51</sup>	201	BM	Phase II	13%	15%	31%	Engraftment: 98% NRM (5 years): 51% OS at 5 years: 19%
2005 <sup>55</sup>	104	PBSC (mega dose)	Phase II	8% (any grade)	7%	26%	Engraftment: 93% DFS at 2 years for AML and ALL in CR: 48% and 46%
2006 <sup>98</sup>	34	PBSC (mega dose)	Phase II	13	12	41%	Engraftment: 91% OS at 2 years: 26%

Abbreviation: BM = Bone marrow; PBSC = peripheral blood stem cell.

**Table 7.** Outcome of studies of  $\alpha\beta$  T cells/CD19 depletion in haplo HSCT

Year published	n	Graft type	Study design	aGVHD (grades II–IV)	Relapse	Other findings
2012 <sup>99</sup>	25	PBSC	Phase II (children with advanced hematological diseases).	36%	N = 13 died with relapsed disease	Engraftment failure (primary): 12% cGVHD: 28% NRM: 16%
2014 <sup>83</sup>	23	PBSC	Phase II (children with non-malignant diseases)	13% (none was grade III or IV)	Not reported (except for graft failure rate of 17%)	Engraftment failure (primary): 17% cGVHD: 0% (18 months follow up) NRM: 9% DFS at 2 years: 91%
2015 <sup>82</sup>	37 (27 MUD, and 10 haplo)	PBSC	Phase II (children with immunodeficiency disorders)	22%	Not reported (except for graft failure rate of 27%)	Engraftment failure (primary and secondary): 27% (salvaged by second transplant) cGVHD: 5% (15 months follow up). NRM: 3% OS at 1 year: 97% Similar outcome of MUD and haplo

Abbreviations: MUD = matched unrelated donor; PBSC = peripheral blood stem cells.

### CD3+/CD19+ AND $\alpha\beta$ T-CELL/CD19+ CELL DEPLETION

The rationale of the initial combined depletion of donor CD3+ T-cell and CD19+ B cells is to eliminate the T cells that mediate GVHD, and B cells that are implicated in EBV-driven post-transplantation lymphoproliferative disorders and possibly decreasing risk of cGVHD as well. Based on initial promising data of CD3+/CD19+ cell depletion at St Jude Children's Research Hospital (Memphis, TN, USA),<sup>56,57</sup> the University of Tubingen group performed clinical studies utilizing this approach with haplo HSCT with resulting risk of grades II–IV aGVHD of 50%.<sup>58,59</sup> One study of haplo HSCT on adult patients (n = 29) utilized CliniMACS device (microbeads CD3+/CD19+ depletion method) without post-transplant immunosuppression<sup>58</sup> and the study reported favorable engraftment and donor chimerism at 1 month post transplant. Grades II–IV aGVHD was observed in 48% and NRM was 20%. The same German group later reported results of a prospective multicenter phase II study using the same method of CD3+/CD19+ cell depletion with haplo HSCT in adult patients (n = 61).<sup>59</sup> In this study, the incidences of grades II–IV aGVHD and

cGVHD were 46 and 18% respectively with NRM at 2 years of 42%. The cumulative incidence of relapse/progression at 2 years was 31%. The OS at 2 years was 28%.

The circulating CD3+ T cells are either  $\alpha\beta$  T cells (95%), or  $\gamma\delta$  T cells (5%).<sup>60</sup> The  $\alpha\beta$  T cells are implicated in adaptive immune response that mediates GVHD, while  $\gamma\delta$  T cells, being part of innate immune system, are not implicated in causing GVHD.<sup>61,62</sup> With the advent of monoclonal antibody depletion technology, interest was directed to depletion methods that only eliminate  $\alpha\beta$  T cells, sparing  $\gamma\delta$  T cells and NK cells.<sup>57</sup> The  $\alpha\beta$  T-cell depletion began with the discovery of T10B9, later known as T10B9.1A-31/MEDI-500, a short-acting non-mitogenic murine immunoglobulin M kappa (IgM $\kappa$ ) mAb directed against the TCR  $\alpha\beta$  complex (discovered by University of Kentucky).<sup>63</sup> The first lot of T10B9 was extracted from mouse ascites and approved by the US FDA (Food and Drug Administration) for T-cell depletion using complement-mediated lysis under BB-IND-4279.<sup>64–66</sup> T10B9 modulates the  $\alpha\beta$  but not the  $\gamma\delta$  TCR, in contrast to OKT3 which binds to the  $\epsilon$  (epsilon) portion of the CD3 receptor and modulates the entire epitope, thus depleting CD3+ T cells (both  $\alpha\beta$  and

$\gamma\delta$  T cells). Immune reconstitution studies revealed that NK cell recovery was significantly greater in patients that received  $\alpha\beta$  TCD grafts than those who received unmanipulated grafts through the first year post transplant.<sup>67</sup> T10B9-based TCD transplant was evaluated in a multicenter BM depletion trial sponsored by the National Heart, Lung and Blood Institute. Although neutrophil recovery, GVHD, grades III–IV toxicities, and hospital days were reduced or improved in the TCD group, CML relapse and CMV reactivation tended to be higher.<sup>68</sup> Sparing of  $\gamma\delta$  T cells allowed transplantation of a partially T-cell depleted marrow graft, which resulted in favorable homeostatic reconstitution of  $\gamma\delta$  T cells in a significant subset of patients compared with that observed with patients receiving OKT3-depleted grafts.<sup>69</sup> Decreased relapse rate was noted among haplo HSCT using  $\alpha\beta$  TCD (T10B9) compared with haplo CD3+ pan TCD (using OKT3).<sup>51</sup> A subset of patients that received haplo  $\alpha\beta$ TCD transplant showed homeostatic reconstitution of increased peripheral blood  $\gamma\delta$  T-cell counts that correlated with showed a significant improvement in relapse-free survival.<sup>66,69</sup> The survival advantage associated with high circulating numbers of  $\gamma\delta$  T cells was found to be durable over seven years following HSCT.<sup>70</sup> These finding implied potential anti-neoplastic activity of the  $\gamma\delta$  T cells. Preclinical and clinical studies have confirmed the anti-neoplastic effect of  $\gamma\delta$  T-cell against hematological malignancies<sup>71–74</sup> as well as other solid tumors.<sup>75–77</sup> The utilization of  $\gamma\delta$  T cells in immunotherapy has been reviewed before.<sup>62</sup> Preservation of  $\gamma\delta$  T cells can also potentially protect against infections.<sup>78,79</sup>

More recently, the CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany) was introduced using immunomagnetic microbead depletion (using the IgG clone BMA-031) with resulting 3–4 log reduction of the  $\alpha\beta$  T cells and B cells.<sup>80</sup> The efficacy of this depletion strategy was tested in in 200 procedures performed over 3 years in one published study.<sup>81</sup> Clinical studies have been performed using  $\alpha\beta$  T-cell/CD19+ B cell depletion approach mainly in the pediatric population in Europe (Table 7).<sup>57,82,83</sup> Notably, one clinical study of pediatric patients with primary immunodeficiency syndromes used  $\alpha\beta$  TCD/CD19+ BCD of HLA-matched unrelated and haplo PBSC transplantation showed favorable T-cell recovery with most patients having peripheral blood T-cell count  $>500/\text{ul}$  by day +120.<sup>82</sup> In this study, the risk of primary or secondary engraftment failure was 27% (all cases salvaged by second transplant), NRM was 3.3% and OS at 1 year was 97%. Another study used similar approach in haplo PBSC HSCT without pharmacological GVHD prophylaxis showed comparable results.<sup>83</sup> Recent findings by Airoidi confirmed the homeostatic reconstitution of  $\gamma\delta$  T cells following  $\alpha\beta$  T-cell/CD19 + B cell depletion in children receiving haplo HSCT.<sup>84</sup>

## FUTURE DIRECTIONS

It is likely the future of transplant therapy will involve more strategies such Chimeric Antigen Receptor (CAR) T-cell therapy, Bi-specific T-cell engagers, and checkpoint inhibitors to control relapse following allo HSCT.<sup>85</sup> The adoptive use of Treg<sup>86</sup> and suicide gene manipulation may improve the risk of GVHD post transplant.<sup>44</sup> Naive T cells (CD45RA+/CD62L+) are mature un-sensitized T cells were shown to be implicated in causing GVHD in preclinical models.<sup>87</sup> A phase II clinical trial showed that selective depletion of naive T cells decreases risk of cGVHD.<sup>88</sup> Other experimental models suggest that different subtypes of functional T cells can be generated from human induced pluripotent stem cells via *in vitro* cellular manipulation laying foundation of potential anti-neoplastic patient-specific T-cell therapy.<sup>89</sup> The utilization of  $\gamma\delta$  T-cells to mitigate the risk of relapse and to enhance immune reconstitution after allo HSCT continued to be under investigation. Results of phase I studies using add-back of  $\alpha\beta$  T-cell- depleted product (following haplo

PBSC transplant with post-transplant cyclophosphamide is awaited (NCT02193880).

## CONFLICT OF INTEREST

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