Allogeneic Stem Cell Transplantation Platforms With Ex Vivo and In Vivo Immune Manipulations: Count and Adjust

Moniek de Witte[1], Laura G. M. Daenen[1], Lotte van der Wagen[1], Anna van Rhenen[1], Reiner Raymakers[1], Kasper Westinga[2], Jürgen Kuball[1,3]

Correspondence: Jürgen Kuball (j.h.e.kuball@umcutrecht.nl).

Abstract
Various allogeneic (allo) stem cell transplantation platforms have been developed over the last 2 decades. In this review we focus on the impact of in vivo and ex vivo graft manipulation on immune reconstitution and clinical outcome. Strategies include anti-thymocyte globulin- and post-transplantation cyclophosphamide-based regimens, as well as graft engineering, such as CD34 selection and CD19/αβT cell depletion. Differences in duration of immune suppression, reconstituting immune repertoires, and associated graft-versus-leukemia effects and toxicities mediated through viral reactivations are highlighted. In addition, we discuss the impact of different reconstituting repertoires on donor lymphocyte infusions and post allo pharmacological interventions to enhance tumor control. We advocate for precisely counting all graft ingredients and therapeutic drug monitoring during conditioning in the peripheral blood, and for adjusting dosing accordingly on an individual basis. In addition, we propose novel trial designs to better assess the impact of variations in transplantation platforms in order to better learn from our diversity of "counts" and potential "adjustments." This will, in the future, allow daily clinical practice, strategic choices, and future trial designs to be based on data guided decisions, rather than relying on dogma and habits.

Neglected basic principles of transplantation: count!

αβT cells are considered to be the major driver of the curative graft-versus-leukemia (GVL) effect, as well as graft-versus-host disease (GVHD), a life-threatening complication that limits the widespread use of allogeneic stem cell transplantations (allo-SCTs).[1–3] Retrospective studies analyzing real world stem cell transplantation data and graft compositions from registries and larger centers suggest that the dose of αβT cells is not well balanced when infused into patients, with a substantial fraction of patients receiving too many αβT cells. The surplus of αβT cells per body weight seems to mainly result in increased incidences of both acute and chronic GVHD, without improving GVL effects or engraftment.[4,5] Within this context, approximately 25% of all individuals in T cell repleted allo-SCT with matched unrelated donors (MUDs)[4] and 50% from haploidentical donors would benefit from infusing fewer donor cells[5] (Figure 1). This observation emphasizes that grafts differ substantially in immune compositions, and these variations need

to be taken into consideration when treating patients. Limiting T cell numbers rarely interferes with stem cell numbers needed for a sufficient engraftment.[4,5] In addition to qualitative and quantitative variations of cell types in the stem cell product, chemotherapeutic drugs used during conditioning can also impact complications and efficacy after allo-SCT. This is a consequence of the fact that concentration of a defined drug, for example, in the blood stream, cannot be precisely predicted based on body weight, body surface area, or kidney or liver function. Active drug levels in the peripheral blood interfere, however, with acute and late toxicity and immune reconstitution, drug dosage needs to be better individualized for patients.[6–10] Therapeutic monitoring chemotherapeutic drugs would allow for the creation of an optimized balance between tumor reduction, space for a new hematopoietic stem cell system, inflammation, as well as immune reconstitution. A first step to overcome inter-individual variations and progress towards the generation of personalized transplantation care was the therapeutic drug monitoring of busulfan, which has been shown to reduce toxicity and has entered clinical practice in many centers across the globe.[6–9] Variations in fludarabine levels have been accounted to impact T cell reconstitutions,[10] and prospective studies are under way to test whether fine-tuning fludarabine levels for each patient will better synchronize immune reconstitution and improve clinical outcomes (NL6940).

Balancing anti-thymocyte globulin in T cell replete and deplete transplantations

Anti-thymocyte globulin (ATG) is a polyclonal antibody composite, raised by animal immunization with human T cells and as such, recognizing many different targets expressed in the hematopoietic system.[11–13] Different types of ATG and different

[1]Department of Hematology, University Medical Center Utrecht, The Netherlands
[2]Cell Therapy Facility, University Medical Center Utrecht, The Netherlands
[3]Center for Translational Immunology, University Medical Center Utrecht, The Netherlands
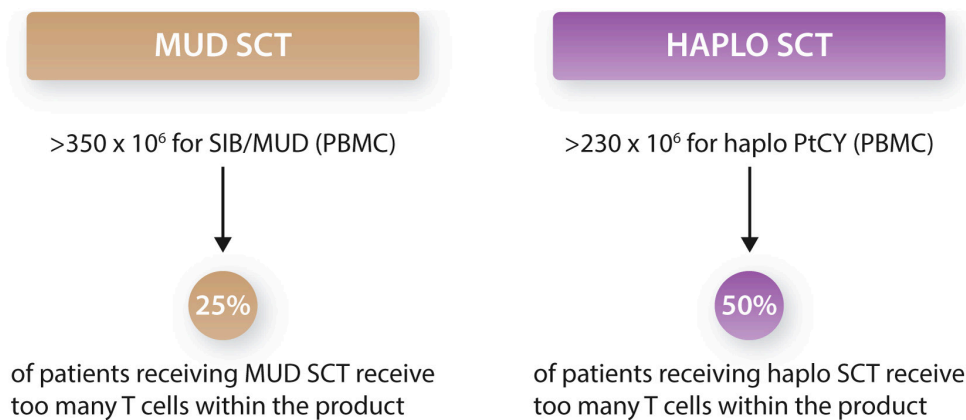
Figure 1. Overdosing of T cells during stem cell transplantation in T cell replete transplantations from matched unrelated and haploidentical grafts. We illustrate different T cell dosage within the context of 2 key studies.[4,5] Haplo = haploidentical donor; MUD = matched unrelated donor; PBMC = peripheral mononuclear cells; PTCy = post-transplantation cyclophosphamide; SCT = stem cell transplantation; SIB = sibling.

batches are currently used world-wide in sibling and in unrelated donor transplantations (eg, ATG-Thymoglobulin and ATG-Fresenius; Table 1 and[14–19]). The impact of clinical outcome differs across the globe. Reduced incidences of GVHD have been reported when ATG was added to conditioning regimens in Europe, which translated into an increased GVHD-relapse free survival.[12] However, ATG did not show improved composite endpoints in US-based prospective clinical trials or retrospective studies.[13] To understand these different clinical outcomes, it is important to acknowledge that the timing of ATG before infusion of the graft is crucial in determining the impact of ATG on the infused graft and subsequent immune reconstitution. When ATG is administered very early before transplantation (eg, from day −12) it mainly acts on host T cells and host-derived antigen presenting cells in order to facilitate engraftment and reduce GVHD by preventing cross-presentation. If ATG is administered shortly before transplantation (from day −7 or later), most ATG types will, because of their rather long half-life, affect the graft. This results in an additional in vivo T cell depletion of the infused stem cell product by circulating active ATG (Figure 2). US-based clinical trials showed no benefit of ATG on relapse and GVHD-free survival after allo-SCT,[19] most likely caused by the usage of irradiation during the conditioning of the patient and ATG administration, which was placed directly after irradiation. Irradiation of patients resulted in a higher in vivo T cell depletion when compared to chemotherapy-based regimens and thus more active ATG remained in the peripheral blood when the graft was infused. Consequently, a stronger donor T cell depletion was accomplished. The net effect was that GVHD was substantially reduced in this clinical trial, but at a cost of substantially more infectious-related deaths.[45] Active ATG at the moment of stem cell infusion is a highly uncontrolled mechanism where inter-individual variations in active ATG levels have been acknowledged as a challenge. Within the context of cord blood transplantations, overexposure of active ATG after allografting has been reported to negatively impact immune reconstitution and clinical outcomes.[46,47] A major development to master these variations in active ATG is the forthcoming practice to determine active levels of ATG, both pre- and post-allo-SCT,[48,49] as well as the effort to generate predictive models to minimize large individual variations in pharmacokinetics (PK) and pharmacodynamics (PD) of ATG.[50] Within this context, inter-individual variations of active ATG have been also reported for a T cell replete reduced intensity conditioning cohort in adult patients to substantially impact clinical outcomes in terms of event-free and overall survival.[51] We have proposed models which indicate that inter-individual variations in active ATG might be overcome

by dosing ATG on lymphocyte counts prior to transplantation instead of on body weight, although this approach needs to be validated for other transplantation regimens and different types of ATG.[52] Despite this rather limited knowledge, recommendations have been suggested by the European Society for Blood and Marrow Transplantation (EBMT) on dosing ATG based on individual lymphocyte counts in order to avoid too deep of a T cell depletion of the patient in the first months after allo-SCT.[53] These insights also have a major impact on T cell deplete transplantations. For example, within the context of graft engineering, where ATG is used to allow a sufficient engraftment of T cell depleted grafts, ATG needs to be given very early before graft infusion (from day −12, thymoglobulin 1.5 mg/kg IV days −12 to −9) in order to prevent further disturbance of the precisely ex vivo designed graft composition.[38] Again, the type of ATG will also impact its timing and dosing of ATG-Fresenius is different (12 mg/kg).[29,39]

Alternative ex vivo and in vivo T cell depletion platforms: campath and post-transplantation cyclophosphamide

To overcome the high variety in ATG products in terms of specificity and numbers used for in vivo T cell depletion, many centers have explored and still use campath. Campath is an anti-CD52 antibody which associates in clinical outcomes with low incidences of GVHD and can be given ex vivo "in the bag" or in vivo (Table 1 and[20–22]). A similar impact on inter-individual variations in lymphocyte counts on active campath in the peripheral blood was reported.[54] However, to the best of our knowledge, there have been no published transplantation studies based on this information. Disease-specific properties also need to be taken into consideration. For example, patients suffering from myelofibrosis have been reported to suffer from higher incidences of GVHD, and adding ruxolitinib before and shortly after transplantation has been reported to dampen GVHD, most likely by reducing cytokine storms.[55,56] Another interesting transplantation platform is in vivo T cell depletion by administration of high-dose post-transplantation cyclophosphamide (PTCy) shortly after transplantation to eradicate allo-reactive αβT cells, which was first reported in transplantations using haploidentical donors. PTCy was also used more recently in HLA-matched donors (Table 1).[23–28] Although PTCy allowed for a rapid increase in the use of haploidentical donors,[57] most recent EBMT registry studies suggest that there is no substantial difference in composite endpoints when

Table 1

Different Types of Transplantation Platforms, Clinical Outcomes, Viral, Infections and Immune Repertoires.

Study	Patients	Donor	Intervention	Numbers (n)	Acute GVHD	Chronic GVHD	EFS/OS	CRFS	Relapse	NRM	CMV	EBV	BK	Adeno
ATG														
Chang et al[14]	Adult hematological malignancies	MRD; PBSC/BM	ATG-T 1.5mg/kg on day −3 to −1	263	II–IV: 13.7% III–IV: 8.4%	2 y 27.9% (ext 8.5%)	3 y OS 69% LFS 65.7%	3 y 38.7%	3 y 20.8%	3 y 9.9%	Day 100: 22.7%	Day 180: 7.8%	NA	NA
Walker et al[15]	Adult hematological malignancies	MUD/MMUD; PBSC/BM	ATG 5 mg/kg on day −2, and 2 mg/kg on days −1 and +1	101	NA	2 y 26.3%	2 y OS 70.6%	1 y 57.6%	2 y 16.7%	2 y 21.2%	NA	20% DNAemia requiring therapy	NA	NA
Socié et al[16]	Adult hematological malignancies	MUD; BM/PBSC	ATG-F 20mg/kg day −3 to −1	103	NA	3 y 12.2%	3 y OS 55%	NA	3 y 33%	3 y 19%	NA	NA	NA	NA
Baron et al[17]	Adult AML	MRD/MUD; PBSC	ATG-F 30mg/kg or ATG-T 5 mg/kg; days not known	569 ATG-fresenius; 249 ATG-thymo-globulin	II–IV: 18%–24% III–IV: 6%–7%;	2 y 30%	2 y OS 67%–68% LFS 60%–61%	2 y 50%–52%	2 y 24%–28%	2 y 11.5%–16%	NA	NA	NA	NA
Finke et al[18]	Adult hematological malignancies	MRD/MUD; PBSC/BM	ATG-F 20mg/kg on day −3 to −1	103	I–IV: 56.3% II–IV: 33% III–IV: 11.7%	2 y 30.8% (ext 12.2%)	2 y OS 59.2% EFS 51.6%	NA	2 y 28.9%	2 y 19.6%	53.8% DNAemia; 5.7% CMV disease	5% PTLD	NA	NA
Soiffer et al[19]	Adult AML, MDS, ALL	MUD; PBSC/BM	ATG-F 20mg/kg −3 to −1	126	II–IV: 23% III–IV: 4.3%	2 y 16% Moderate-severe 12%	2 y OS 59% PFS 47%	2 y 38%	2 y 32%	2 y 21%	62% (R+) DNAemia	1.6% PTLD	NA	NA
Alemtuzumab														
Green et al[20]	Adult hematological malignancies	Matched/mis-matched; PBSC/BM	Alemtuzumab dose 50–100 m	313	II–IV: 32%–40% III–IV: 2%–15%	2 y 32%–41%	NA	NA	2 y 24%–36%	2 y 16%–24%	>80% (R+) DNAemia	NA	NA	NA
van Besien et al[21]	Adult AML/MDS	MRD/MUD/MMUD PBSC/BM	Alemtuzumab	95	II–IV: 23.3% III–IV: 8.6%	2 y 16%	2 y OS 40.5% PFS 33%	NA	1 y 23.7%	1 y 24.6%	NA	NA	NA	NA
Carpenter et al[22]	Adult hematological malignancies	MRD/MMRD/MUD/ MMUD; PBSC/BM	Alemtuzumab (75 in vivo, 36 ex vivo)	111	NA	NA	NA	NA	NA	NA	NA	2 y 40.3% DNAemia; 1% PTLD	NA	NA
PTCy														
Kanakry et al[23]	Adult leukemia/MDS	MRD/MUD; BM	PTCy	209	II–IV: 45% III–IV: 11%	3 y 13%	3 y OS 58% EFS 46%	3 y 39%	3 y 36%	3 y 17%	NA	NA	NA	NA
Cieri et al[24]	Adult high-risk hematological malignancy	Haplo; PBSC	PTCy	40	II–IV: 15% III–IV: 7.5%	1 y 20%; severe 5.1%	1 y OS 56% EFS 48%	NA	1 y 35%	1 y 17%	63% DNAemia 17% CMV disease	15% DNAemia (66% 18% of these pts treated). No PTLD	NA	NA
Berger et al[25]	Pediatric; high-risk hematological malignancy	Haplo; BM/PBSC	PTCy	33	II–IV: 22% III–IV: 3%	1 y 4%	1 y OS 72% EFS 61%	NA	1 y 24%	1 y 9%	36% DNAemia No CMV disease	3% DNAemia No PTLD	17%	3% DNAemia; not symptomatic
Devillier et al[26]	AML/high-risk MDS	Haplo; BM/PBSC	PTCy	60	II–IV: 18% III–IV: 2%	2 y 14%; severe 4%	1 y OS 50% EFS 39%	1 y 37%	1 y 34%	1 y 27%	NA	NA	NA	NA
Mehta et al[27]	Adult hematological malignancies	MMUD; BM/PBSC	PTCy	41	II–IV: 37% III–IV: 17%	2 y 30%	2 y OS 52% EFS 42%	NA	2 y 20%	2 y 35%	NA	NA	NA	NA
Mielcarek et al[28]	Adult hematological malignancies	MRD/MUD/MMUD; PBSC	PTCy	43	II–IV: 77% III–IV: 0%	1 y 16% requiring IS; ext 30%	2 y OS 70% 2 y EFS 69%	2 y 50%	2 y 17%	2 y 14%	NA	NA	NA	NA

(Continued)

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Different Types of Transplantation Platforms, Clinical Outcomes, Viral, Infections and Immune Repertoires.

Study	Patients	Donor	Intervention	Numbers (n)	Acute GVHD	Chronic GVHD	EFS/OS	CRFS	Relapse	NRM	CMV	EBV	BK	Adeno
PTCy vs ATG														
Battipaglia et al[29]	Adult AML	MMUD; BM/PBSC	PTCy vs ATG-F/ATG-T	93 PTCY, 179 ATG	PTCY II–IV: 30%, III–IV: 9%; ATG: II–IV: 32%, III–IV: 19%	PTCY Any: 39%, Ext: 17%; ATG Any: 36%, Ext: 20%	PTCY 2 y OS: 56%, 2 y EFS: 55%; ATG 2 y OS: 38%, 2 y EFS: 34%	PTCY: 2 y 37%, ATG: 2 y 28%	PTCY: 2 y 29%, ATG: 2 y 37%	PTCY: 2 y 16%, ATG: 2 y 29%	NA	NA	NA	NA
Battipaglia et al[30]	Adult AML	MRD; BM/PBSC	PTCy vs ATG-F (26%)/ATG-T (74%)	197 PTCY, 1913 ATG	PTCY II–IV: 19%, III–IV: 6%; ATG: II–IV: 17%, III–IV: 6%	PTCY Any: 37%, Ext: 16%; ATG Any: 30%, Ext: 12%	PTCY 2 y OS: 64%, 2 y PFS: 55%; ATG 2 y OS: 65%, 2 y PFS: 58%	PTCY: 2 y 44%, ATG: 2 y 49%	PTCY: 2 y 36%, ATG: 2 y 32%	PTCY: 2 y 8%, ATG: 2 y 10%	NA	NA	NA	NA
Brissot et al[31]	Adult AML	MUD; BM/PBSC	PTCy vs ATG-T 5mg/kg	174 PTCY, 1452 ATG	PTCY II–IV: 28.8%, III–IV: 8.8%; ATG: II–IV: 29.2%, III–IV: 9%	PTCY Any: 31.4%, Ext: 18.5%; ATG Any: 33.6%, Ext: 13.1%	PTCY 2 y OS: 62.7%, 2 y PFS: 59.7%; ATG 2 y OS: 64.8%, 2 y PFS: 59.6%	PTCY: 2 y 41.6%, ATG: 2 y 49.3%	PTCY: 2 y 25.2%, ATG: 2 y 23.7%	PTCY: 2 y 15.2%, ATG: 2 y 16.7%	NA	NA	NA	NA
Ruggeri et al[32]	Adult AML	MUD; BM/PBSC	PTCy vs ATG-F/ATG-T	193 PTCY, 115 ATG	PTCY III–IV: 4.7%; ATG: III–IV: 12.5%	PTCY Any: 33.7%, Ext: 8.6%; ATG Any: 28.3%, Ext: 12.6%	PTCY 2 y OS: 58%, 2 y PFS: 56%; ATG 2 y OS: 54.2%, 2 y PFS: 47.2%	PTCY: 2 y 50.9%, ATG: 2 y 38.9%	PTCY: 2 y 21.6%, ATG: 2 y 22.3%	PTCY: 2 y 22.4%, ATG: 2 y 30.5%	NA	NA	NA	NA
Retière et al[33]	Adult hematological malignancies	MRD/MUD/MMUD/haplo; BM/PBSC	PTCy vs ATG-T 2.5mg/kg on day −1 or days −2 and −1	30 PTCY, 15 ATG	PTCY II–IV: 47%, III–IV: 10%; ATG: II–IV: 47%, III–IV: 20%	NA	PTCY 2 y OS: 79%, 2 y PFS: 63%; ATG 2 y OS: 73%, 2 y PFS: 60%	NA	PTCY: 2 y 17%, ATG: 2 y 33%	NA	DNAemia PTCY 27% ATG 40%	DNAemia requiring treatment PTCY 0% ATG 33%	PTCY 3% ATG 0%	PTCY 15% ATG 20%
Ex vivo graft engineering														
Pasquini et al[34]	Adult AML	MRD; PBSC	CD34+ T cell depletion	44	II–IV: 23%, III–IV: 4.5%	2 y 19%	2 y OS 59% EFS 55%	2 y 42%	2 y 24%	2 y 21%	NA	NA	NA	NA
Barba et al[35]	Adult leukemia	MRD/MUD/MMUD; PBSC	CD34+ T cell depletion	241	II–IV: 16%, III–IV: 5%	3 y 5%	3 y OS 57% EFS 54%	1 y 65% 3 y 52%	3 y 22%	3 y 24%	NA	NA	NA	NA
Bayraktar et al[36]	Adult AML	MRD/MUD/MMUD; PBSC/BM	CD34+ T cell depletion	115	II–IV: 5%, III–IV: 1%	3 y 13%	1 y OS 68% EFS 62% 3 y OS 57% EFS 58%	NA	1 y 17% 3 y 18%	1 y 18% 3 y 24%	NA	NA	NA	NA
van Esser et al[37]	Adult hematological malignancies	SIB/MUD; BM/PBSC	CD34+ T cell depletion or sheep erythrocyte rosetting	85	II–IV: 57%	1 y 38%	NA	NA	NA	1 y 29%	NA	54% (R+) DNAemia 12% PTLD	NA	NA
de Witte et al[38]	Adult hematological malignancies	MRD/MUD/MMUD; PBSC	αβT cell depletion	35	II–IV: 37%, III–IV: 17%	2 y 23% Moderate: 17%	2 y OS 52% EFS 40%	NA	2 y 29%	2 y 32%	64% (R+) DNAemia, CMV disease 6%	44%	NA	NA

(Continued)

Table 1. (Continued)

Different Types of Transplantation Platforms, Clinical Outcomes, Viral, Infections and Immune Repertoires.

Study	Patients	Donor	Intervention	Numbers (n)	Acute GVHD	Chronic GVHD	EFS/OS	CRFS	Relapse	NRM	CMV	EBV	BK	Adeno
Locatelli et al[39]	Pediatric AML/ALL	Haplo; PBSC	αβT cell/CD19 depletion	80	I–II skin only: 30% >II or visceral: 0%	0%	5 y: OS 72% LFS 71%	NA	5 y 24%	5 y 5%	NA	NA	NA	NA
Laberko et al[40]	pediatric malignant (114) + nonmalignant (68)	MUD/haplo; PBSC	αβT cell/CD19 depletion	182	Malignant: II–IV: 40% Nonmalignant II–IV: 27%	NA	2 y OS 68% 2 y malignant 58% 2 y nonmalignant 78%	NA	2 y 37%	2 y 13%	51%	33%	NA	NA
Lang et al[41]	Pediatric AML/MDS/ nonmalignant	Haplo; PBSC	αβT cell/CD19 depletion	41	II: 10% III–IV: 15%	1.6 y average FU: 18% limited; 9% ext	1.6 y average FU: OS 52%; EFS NA	NA	1.6 y average FU: 42%	1.6 y average FU: 7%	NA	NA	NA	NA
Maschan et al[42]	Pediatric high-risk AML	MUD/MMUD/haplo; PBSC	αβT cell/CD19 depletion	33	II: 23% III: 16% IV: 0%	2 y 30%	2 y OS 67% EFS 60%	NA	2 y 31%	2 y 10%	52% DNAemia; 6% CMV disease	50% DNAemia; 6% rituximab	NA	NA
Bertaina et al[43]	Pediatric nonmalignant	Haplo; PBSC	αβT cell/CD19 depletion	23	I–II: 13.1% III–IV: 0%	0%	2 y OS 91% EFS 74%	NA	NA	9.3%	38% DNAemia CMV/adeno	50% DNAemia; 6% rituximab	NA	38% DNAemia CMV/adeno
Balashov et al[44]	Pediatric nonmalignant	MUD/MMRD/haplo; PBSC	αβT cell/CD19 depletion	37	II: 21.5% IV: 2.8%	Any 3% Ext 3%	2 y OS 96.7% EFS 67.7%	NA	NA	NA	NA	NA	NA	NA

Depicted is a selection of studies.

Adeno = adenovirus; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ATG = anti-thymocyte globulin; ATG-F = anti-thymocyte globulin-fresenius; ATG-T = anti-thymocyte globulin-thymoglobulin; BK = BK virus; BM = bone marrow; CMV = cytomegalovirus; CRFS = cGVHD-free relapse-free survival; cGVHD = chronic graft vs host disease; EBV = Epstein-Barr virus; EFS = event-free survival; ext = extensive; FU = follow up; GVHD = graft vs host disease; haplo = haploidentical donor; IS = immune suppression; LFS = leukemia-free survival; MDS = myelodysplastic syndrome; MMRD = mismatched related donor; MMUD = mismatched unrelated donor; MRD = matched related donor; MUD = matched unrelated donor; NA = not available; NRM = nonrelapse mortality; OS = overall survival; PBSC = peripheral blood stem cell; PFS = progression-free survival; PTCY = post-transplantation cyclophosphamide; PTLD = posttransplant lymphoproliferative disease; pts = patients; R+ = cytomegalovirus positive recipient; SIB = sibling; y = year.
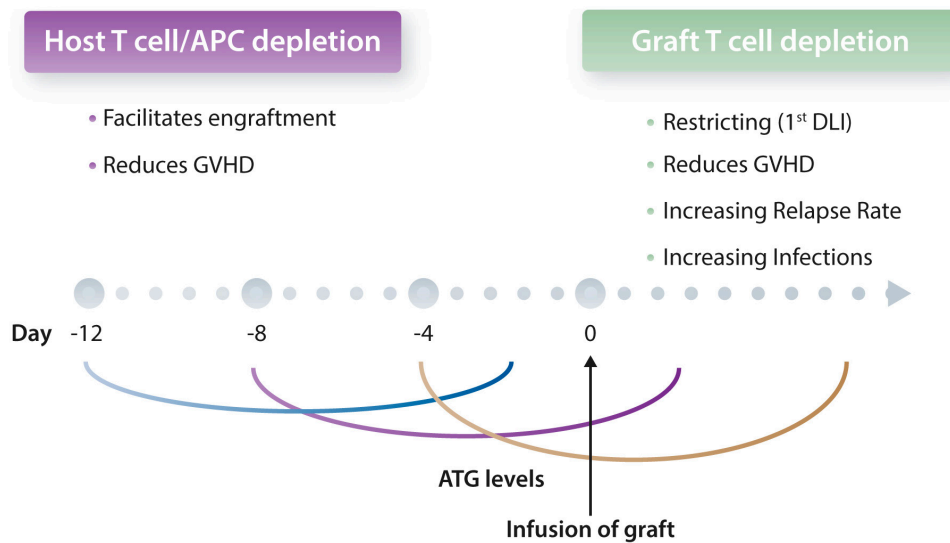
Figure 2. The dual sword of ATG, it is all about timing. APC = antigen presenting cell; ATG = anti-thymocyte globulin; DLI = donor lymphocyte infusion; GVHD = graft vs host disease.

comparing ATG to PTCy in MUD donors (Table 1).[29–33] Given that PTCy will be difficult to individualize in terms of PK and PD because of its complex chemistry,[58] based on the retrospective analysis of registry data, one could even argue that dose adjusted ATG[45,51] might be the preferred choice to date in fully matched MUD donors, while PTCy appears to be superior when 9 out of 10 MUD donors[29] or haploidentical donors[32] are used.

Ex vivo T cell engineering strategies focusing on defined subsets

Ex vivo T cell engineering by means of CD34 selection (Table 1) [34–37] or αβ T cell depletion (Table 1) [38–44] is the most controlled way to define graft composition to date, and has been shown to be at least as successful as other optimized platforms in reducing the incidence of GVHD while maintaining graft-versus-leukemia effects (Table 1). Pasquini et al[34] reported that CD34 selection of peripheral derived blood stem cells of HLA-matched sibling donors results in a well-defined allograft, with $0.01–1 \times 10^5$ αβ T cells/kg, and is associated with a low incidence of chronic GVHD. Subsequently, αβ T cell/CD19 depletion entered clinical practice in haploidentical-SCT,[59] matched related donors, and MUD.[42] These depletion techniques restrict αβ T cells to around $0.1–1 \times 10^5$ αβ T cells/kg[59] (comparable with CD34 selection) while preserving natural killer (NK) cells (CD3 depletion) or NK and γδ T cells (αβ T cell depletion). αβ T cell depletion associated with a low incidence of acute GVHD in a cohort of pediatric patients with both malignant and nonmalignant diseases[43,60] was reported also for PTCy (Table 1). In a very recent study of adult patients with malignant disease, with a longer follow-up, chronic GVHD rate was also surprisingly low.[38] Because graft engineering is more expensive and cumbersome compared to the application of ATG or PTCy, at this stage, most centers use ATG- or PTCy-based regimens. However, as engineering chimeric antigen receptor T cells increases in availability and prevalence, the complexity and costs of graft engineering will become negligible for centers when assessing its potential strategic advantages.[3]

The use of more complex engineering techniques will largely depend on reimbursement strategies. Beginning in 2020 in the Netherlands, reimbursement for graft engineering should facilitate its implementation to a broader patient population. In addition, as randomized studies comparing different transplantation platforms strategies will either not be performed at a larger scale, or are quickly outdated given the rapid pace of developments, creation of a new network of studies, like those designed for coronavirus disease (COVID)-related research, seems to be timely.[61] The EBMT registry covering transplantations and cellular immune therapies like CAR T cells will be well-suited to serve as a potential backbone for these studies, but would need to be extended.[62,63] These studies should also include inquiries into the socio-economic impacts and quality of life for patients in order to cover all aspects of earlier financial investments and future societal gains.[62]

T cell depletion and viral reactivations

The 2 clinical measurements of a well-balanced T cell reconstitution are the incidence of GVHD and viral reactivations. While data on incidence of GVHD are usually decently reported in registries and clinical trials, data on different viral reactivations or infections after allo-SCT in the different platforms are scarce (Table 1). Lack of reporting does not necessarily indicate the absence of the event. For example, initial reports on PTCy did not report on BK virus (BK)-reactivation, while later studies indicate that BK-reactivations are a substantial clinical problem in up to one fifth of all patients.[24,25] Also, reporting on cytomegalovirus (CMV) reactivations is rather heterogenous. While incidences of infections are related to the overall cohort (frequently around 30%–40%) most of the time, incidences can be underestimated, as reactivation occurs mainly in CMV-positive recipients. Reporting incidence in relation to recipient positivity would be more appropriate[38] and allow for a better comparison of incidences between different trials and retrospective cohorts. The difference between preemptive CMV treatment and actual CMV disease is important information for assessing the strength of an immune system and more important than diving into different techniques and thresholds of CMV reporting, which are rather heterogenous and do not inform us about immune competence.[64] Incidences on Epstein-Barr virus (EBV) infections and reactivations are also not indicated in most reports (Table 1). Initial studies in 2001 showed an incidence of EBV reactivation of around 30% in T cell replete transplantation platforms, while after CD34 graft engineering with different techniques, 65% of EBV reactivations were reported.[37] EBV reactivations were, at the time of the initial reports, a substantial clinical problem, as anti-CD20 antibodies were only approved in 1998 by the European Medicines Agency. In 2020, the use of ATG or

haploidentical donors were still identified as risk factors for EBV reactivations.[65] Also in the absence of CD19 depletion within the context of an αβT cell depletion, incidences of EBV reactivation have been reported to be around 40%,[38] frequencies which are in line with what was reported after campath conditioning.[22] However, as these reports are from patients treated after anti-CD20 antibodies were approved, no substantial increases in posttransplant lymphoproliferative disorders are observed in either platform to date. Thus, reactivation of a virus does not necessarily affect clinical outcomes, as evidenced by the history of EBV reactivation over the last 2 decades. Adding CD19 depletion during graft engineering to the αβT cell depletion platform, however, nearly abolished EBV reactivations in an interim analysis of an ongoing clinical trial (NL6940, de Witte and Kuball, unpublished observation, 2021). Thus, filling the knowledge gap on viral reactivations in relation to the transplantation regimen in a registry would allow for better assessment of immune competence across different platforms. Viral reactivations do not always need to be harmful, and some viral reactivations might be even beneficial. For example, it has been suggested that CMV reactivations could assist in certain, but not all, transplantation platforms to improve leukemia control.[64,66–71] Novel drugs that prevent CMV reactivation and have been introduced recently to the market[72] could, within this context, be counterproductive for certain transplantation platforms.

Immune reconstitution impacts toxicity and efficacy of post-transplantation viral reactivations and maintenance therapies

Acknowledging that different transplantation platforms associate with different immune repertoires early after transplantation[1] increases our understanding of the different biological impacts of CMV reactivation on toxicity and leukemia control.[68] These different immune repertoires can also provide a strategic reason to choose 1 transplantation platform over the other, as different options arise for maintenance therapies after transplantation. αβ T cell depletion is, in contrast to all other platforms, dominated by an early NK cell and γδT cell recovery, most likely derived from the infused product (Figure 3),[38,39,43,60] which usually does not require immune suppression beyond 1 month. ATG-based platforms favor a strong recovery of αβT cells, which associate with the need of
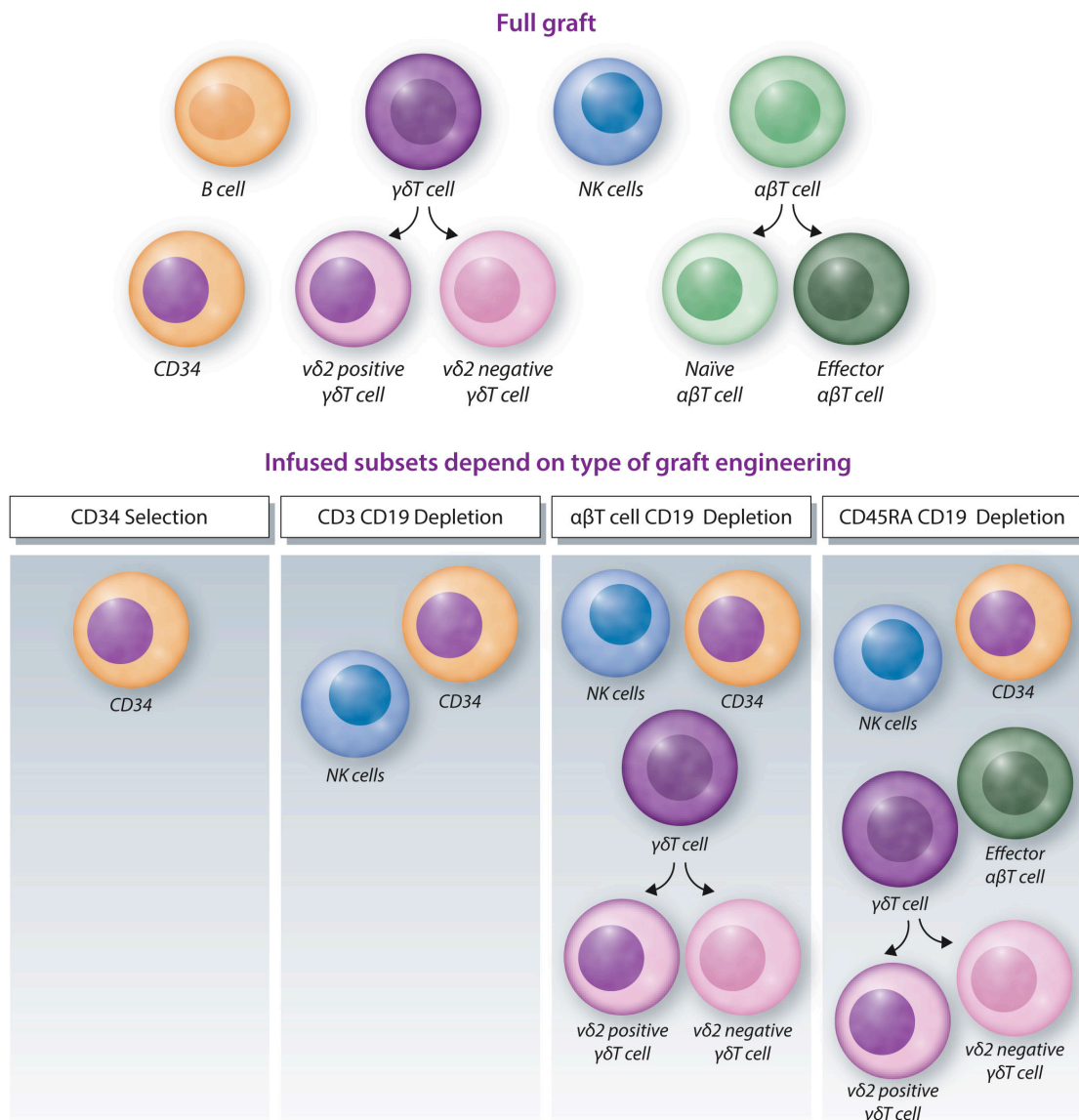

Figure 3. Graft engineering. Different graft compositions are depicted from different graft sources after different types of graft engineering. NK = natural killer.

additional immune suppression for at least 4 months.[51] PTCy has been suggested to selectively deplete rapidly proliferating allo-reactive αβT cells. Rapidly reconstituting NK cells and γδT cells are most likely also diminished as collateral damage of PTCy. However most recent data suggest that depletion of allo-reactive αβT cells is less important than creating a αβT cell dysfunction and suppression.[73,74] This would explain why a prolonged immune suppression is needed after PTCy. The increased immune suppressive environment after PTCy would also better explain the conflicting data reported on numbers between different immune reconstitution patterns observed after ATG and PTCy-based transplantation regimens.[33,75] The described substantial differences in immune reconstitution patterns depending on transplantation regimens most likely explain the aforementioned conflicting data on the impact of CMV reactivation on leukemia control. NK and γδT cells, particularly after αβT cell depletion, are dominant subsets that will be protective as they both expand upon CMV reactivation and also cross-react with leukemic cells.[67,68,76–82] We, and others, have shown that ex vivo T cell depletion strategy regimens favor memory cells like NK cells and γδT cells.[38,39,60] However, a major challenge remains sporadic reporting and lack of harmonization in immune monitoring (eg, Table 2 and,[25,38,39,41,42,83] for review[53]).

Not only infections, but drugs can also act differently, depending on the current immune environment (Figure 4). After T cell replete transplantation, lenalidomide has detrimental effects by inducing lethal GVHD if given shortly after transplantation.[84] In contrast, tyrosine kinase inhibitors, currently used in clinical trials as maintenance therapies for leukemia control, most likely do not substantially depend on the transplantation platform in terms of toxicity. However, part of their activity was suggested to be mediated via interleukin-15 on CD8 positive αβT cells,[85] and a strong immune suppression might therefore impair efficacy. However, more data are needed to thoroughly investigate the impact of post-transplantation pharmacological interventions on clinical outcomes. Checkpoint inhibitors are another example of a drug where the currently available reports suggest that they are most likely harmful if administered too early after transplantation in T cell replete platforms. Toxicity in T cell deplete platforms might be less due to the reduced size of the αβT cell repertoire (Figure 4).[2,86] Also the choice of bispecific molecules could be driven by the transplantation platform. Anti-CD3 engagers could bare the risk of inducing GVHD if used too early in T cell replete platforms, and trispecific killer engager molecules, which mainly act in NK cells, could be the preferred choice for transplantation platforms favoring a strong NK cell reconstitution.[87]

Donor lymphocyte infusions and engineered immune effector cells within the context of different transplantation platforms

Donor lymphocyte infusion (DLI) are considered as the cornerstone of allo-SCT, either used as prophylactic intervention (thus in patients with a priori high risk of relapse) preemptively (thus triggered by minimal residual disease or a reduced donor chimerism), or as therapy once relapse occurs.[1,2] In particular, the preemptive and prophylactic usage of DLIs depends on the transplantation platform in terms of timing and dosing (Figure 5) (modified and updated from Yun and Waller[88]). After αβT cell depletion, we have been able to administer a DLI to the majority of patients 3 months after allo-SCT, while preemptive or prophylactic DLI after other types of transplantations are usually administered later, and for T cell replete transplantations are only an option for a minority of patients (for review see[1]). T cell replete platforms including ATG and PTCy require per default ongoing immune suppression for many months to keep co-infused graft T cells under control. In the case of GVHD, immune suppression needs to be prolonged. However, the drawback of acute GVHD is extensive chronic GVHD in a majority of patients, at least when treated with ATG, and will severely impact quality of life and overall survival. An additional important consideration is the upcoming combination therapy of allo-SCT beyond DLI, but with genetically modified T, NK, γδT cells.[3,77,89–93] These strategies favor platforms that do not depend on prolonged immune suppressions.

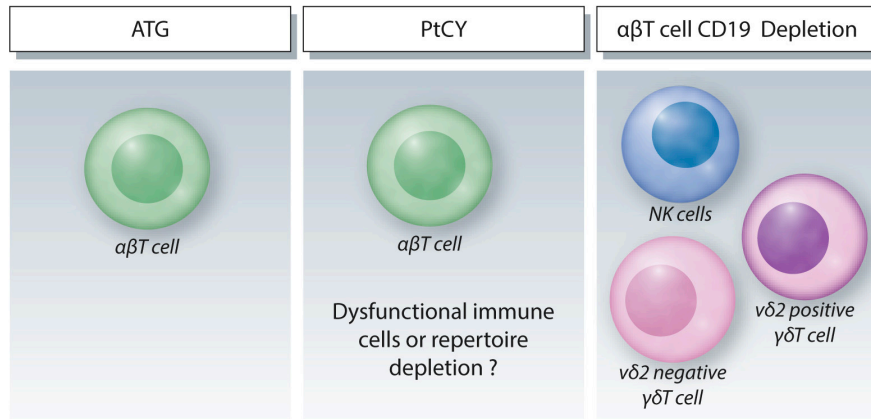**Immunological Recovery After Allo-SCT With T Cell Depletion.**

Study	Intervention	T Cell IR	γδT Cell IR	αβT Cell IR	B Cell IR	NK Cell IR
Berger et al[25]	PTCy	Day 60 CD4 132/µL CD8 491/µL	NA	NA	Day 60 46/µL	Day 60 154/µL
de Witte et al[38]	αβT cell depletion		Day 100 43/µL vd2+ 21/µL vd2- 22/µL	Day 100 CD8 148/µL; CD4 60/µL	Day 100 130/µL	Day 100 182/µL
Locatelli et al[39]	αβT cell/CD19 depletion	Day 100 CD3 254/µL CD4 89/µL CD8 98/µL	Day 100 46/µL	Day 100 186/µL	Day 100 2/µL	Day 100 196/µL
Lang at[41]	αβT cell/CD19 depletion	Day 90 CD3 374–479/µL CD4 120–159/µL	NA	NA	Day 90 24–83/µL	Day 30 352–430/µL
Maschan al[42]	αβT cell/CD19 depletion	Day 30 CD3 245/µL CD4 40/µL CD8 135/µL	NA	NA	NA	Day 30 385/µL
Laberko et al[83]	αβT cell/CD19 depletion	Day 120 CD3 220–384/µL	Day 120 38/µL	Day 120 262/µL	Day 120 18–44/µL	NA

Examples representing diversity in analyses.

allo-SCT = allogeneic stem cell transplantation; IR = immune reconstitution; NA = not available; NK = natural killer; PTCy = post-transplantation cyclophosphamide; µL = microliter.

Dominant immune subsets depend early after transplantation on type of graft depletion

ATG	PtCY	αβT cell CD19 Depletion
αβT cell	αβT cell	NK cells / vδ2 negative γδT cell / vδ2 positive γδT cell
	Dysfunctional immune cells or repertoire depletion ?	

Potential risk of GVHD by post allo SCT maintenance therapies

	ATG	PtCY	αβT cell CD19 Depletion
Kinase inhibitors	Low risk	Low risk	Low risk
Lenalidomide	High risk	Intermediate risk	Intermediate risk
Checkpoint inhibitor	High risk	Intermediate risk	Intermediate risk

Figure 4. Transplantation platforms, associated dominant immune repertoires early after transplantation, and potential risk of GVHD induced by maintenance therapy. ATG = anti-thymocyte globulin; GVHD = graft vs host disease; NK = natural killer; PTCy = post-transplantation cyclophosphamide; SCT = stem cell transplantation.

Conclusions: adjust!

Substantial variations in terms of immune reconstitution are observed within and between different allo-SCT platforms. However, it is striking that the vast majority of allo-SCT studies only report clinical outcomes as parameters of success, and that information on the actual "drug delivered"—the infused donor cells and their capacity to execute a GVL reaction—is often lacking. Better capturing, understanding, and mastering a well-balanced immune recovery for all platforms will be key for increasing
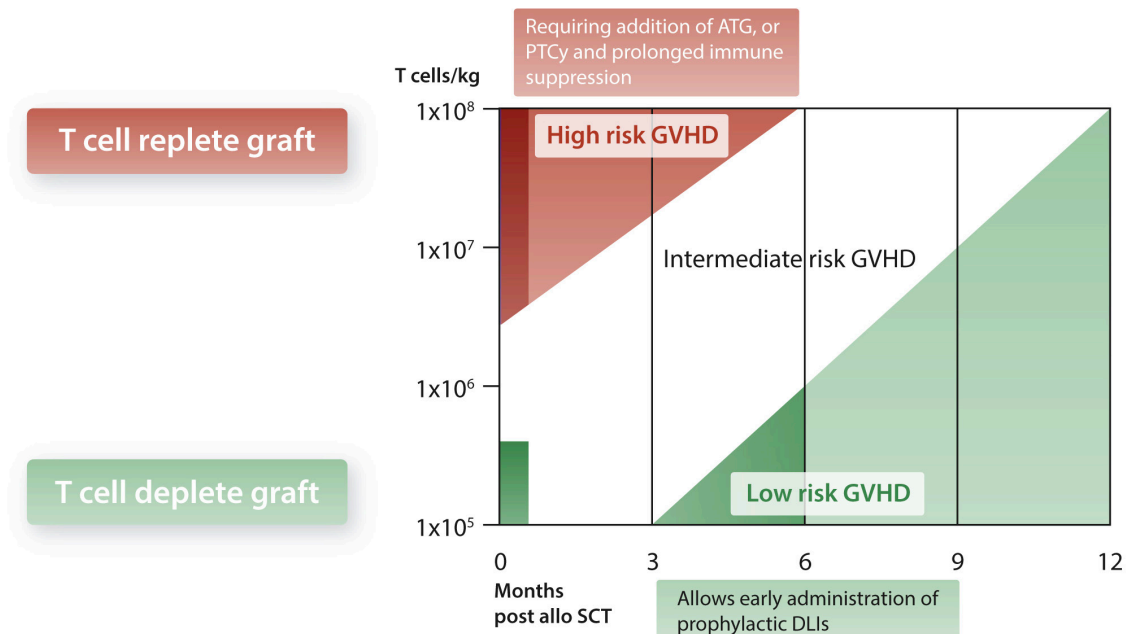

Figure 5. Dosing and timing of DLI. Risk of GVHD is plotted against T cell numbers received either during graft infusion, or later as donor lymphocyte infusion (modified and updated from Yun and Waller[88]). ATG = anti-thymocyte globulin; DLI = donor lymphocyte infusion; GVHD = graft vs host disease; PTCy = post-transplantation cyclophosphamide; SCT = stem cell transplantation.

the overall success of transplantation, as well as for novel post-transplantation interventions. This knowledge will allow for better assessment of the habits and strategic choices of different centers, can guide daily practice, and create a basis for novel trial designs. The benchmarking initiative started by EBMT can assist in increasing data completeness and quality[63] to develop COVID-inspired trial designs such as "a randomized embedded multifactorial adaptive platform"[61] for the entire community to create more data evaluating "counts" and "adjustments."

Practical advice of count and adjust

One might argue that our proposed strategy of individualized allo-transplantation platforms and counting of all graft ingredients is either not applicable in daily routine due to its complexity, or should be first further evaluated in clinical studies. While clinical studies are, of course, of great importance for future insights into individualized transplantation platforms, we provided vast evidence that many steps should be introduced today into clinical routines. We advocate for 4 different pillars, with different levels of readiness. The first pillar, which is ready for all centers today, is overcoming the vast overshoot of T cells in grafts for all T cell replete transplantations. This would only require the addition of CD3 to CD34 counts as a quality control before infusion at a center, as not all donor centers provide grafts with T cell counts (Figure 1). Donor centers are at the same time encouraged to add CD3 counts to CD34 counts during their daily routine. The second pillar is adjusting the dosing of ATG based on lymphocyte counts when using ATG-Thymoglobulin, as advised in the recent EBMT handbook.[53] In contrast, more data will be needed for lymphocyte count-adjusted ATG-Fresenius before providing specific advice. The third pillar is graft engineering as an additional intervention that can be introduced into daily clinical practice if strategically interesting, although associated with expertise of the stem cell laboratory and also with additional costs during the beginning of the transplantation. Whether graft engineering is superior as compared to T cell replete transplantation strategies when carefully restricting dosing of T cells and ATG remains to be clinically studied. The fourth pillar is therapeutic drug monitoring during conditioning, which is well-established for busulfan. Dose adjustments of additional chemotherapy agents used during conditioning have been studied less extensively and need further evaluation before being implemented into daily clinical routine. Thus, we conclude that the 2 main points of advice, either infusing less graft cells and when administering ATG-Fresenius adjusting timing, or dosing for patients with low lymphocyte counts, can be implemented at all centers today without any additional costs.

Disclosures

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