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REVIEW ARTICLE

Contemporary haploidentical stem cell transplant strategies in children with hematological malignancies

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Abstract

The barriers to HLA-mismatched or haploidentical hematopoietic stem cell transplantation (HSCT), namely GvHD and graft failure, have been overcome with novel transplant platforms. Post-transplant Cyclophosphamide (PTCy) is widely available, feasible and easy to implement. TCRαβ T and B cell depletion comes with consistent GvHD preventive benefits irrespective of age and indication. Naive T-cell depletion helps prevention of severe viral reactivations. The Beijing protocol shows promising outcomes in patients with poor remission status at the time of transplantation. For children, the toxicities and late outcomes related to these transplants are truly relevant as they suffer the most in the long run from transplant-related toxicities, especially chronic GvHD. While comparing the outcomes of different Haplo-HSCT approaches, one must understand the transplant immunobiology and factors affecting the transplant outcomes. Leukemia remission status at the time of conditioning is a consistent factor affecting the transplant outcomes using any of these platforms. Prospective comparison of these platforms lacks in a homogenous population; however, the evidence is growing, and this review highlights the areas of research gaps.

Introduction

The haploidentical donor shares one HLA haplotype or a single identical copy of chromosome 6 with a recipient. The family donor may be more than 5/10 HLA match and have common alleles on the unshared haplotype (mismatched related donor). Haplo or mismatched related donor grafts are increasingly being used for allogeneic hematopoietic stem cell transplantation (HSCT) due to a lower risk of graft-versus-host disease (GvHD) and graft failure (GF) with innovative Haplo-HSCT platforms. The modern haplo platforms utilize one of the following principles: (1) in vivo attenuation of allogeneic T cell effects [post-HSCT Cyclophosphamide (PTCy)] (2) ex-vivo depletion of GvHD causing T-cell subsets from graft while retaining beneficial cell subsets [TCRαβ T-cell depletion, naive T-cell depletion]. (3) Modulation of T-cell alloreactivity by using the GIAC approach [G, donor treatment with granulocyte colony-stimulating factor; I, intensified immunological suppression; A, antihuman thymocyte immunoglobulin (ATG) in conditioning; C, a combination of peripheral blood and bone marrow as a graft source]. A refined version of this protocol is known as the "Beijing Protocol". For this review, these platforms are designated A (PTCy), B (TCRαβ T and B cell depletion), C (naive T-cell depletion), and D (Beijing Protocol). Perugia group also pioneered a Haplo approach incorporating an intensified conditioning and administering megadose T-cell-depleted grafts without additional post-HSCT immunosuppression and showed promising outcomes in adults [1, 2]. The use of this approach in children showed a significant relapse rate and mortality [3]. Due to uncommon use in children, this approach will not be discussed.

Novel Haplo-HSCT methods have improved access to HSCT as the availability of haplo donors is nearly universal, and good outcomes can be achieved using not only first-degree but also second degree related [4] or unrelated HLA-mismatched donors [5]. The degree of mismatch also does not negatively affect the transplant outcomes with the use of these platforms [6–8]. Besides the graft-versus-leukemia (GvL) effect mediated by alloreactive HLA-mismatched donor cells, other advantages include a broadened donor pool, motivated donors, rapid and economical graft

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Table 1 Comparison of novel haploidentical transplant platforms.

	PTCy (A)	TCRαβ TCD (B)	Naive TCD (C)	Beijing protocol (D)
Conditioning	MAC or RIC	MAC	MAC or RIC[a]	MAC
Serotherapy	Not needed	Low dose	Not needed	Full dose
Graft source	BM or PBSC	PBSC	PBSC	[b]BM and PBSC
G-CSF use post-HSCT	Yes	No	Yes	Yes
Post-HSCT GvHD prophylaxis	CNI + MMF	None	CNI or sirolimus for 6–8 weeks.	CNI + MMF + MTX
aGvHD risk	++	+ (visceral GvHD rare)	+++ (steroid responsive)	+++
cGvHD risk	++[c]	+	++	+++
Viral infection risk	++[d]	+++	+	++
Graft failure risk	~10–15%[e]	<10%[f]	<10%	<5%
NK cell activity early post-HSCT	Minimal	Preserved[g]	Minimal	Likely suppressed
GvL or anti-infective effect early post-HSCT	Activated T and T_{mem} cells	γδ T and NK cell	T_{mem} cells	Activated T and NK cells
Upfront cost	+	++++	++++	++
A situation where it may be a preferred platform	Resource limited settings, patient's inability to tolerate MAC	Patient with pre-existing GvHD from first HSCT (very high GvHD risk)	Persistent systemic viral infection before HSCT	Poor remission or refractory disease before HSCT

[a]St Jude Study conditioning regimen: total lymphoid radiation (8 Gy), Cy, fludarabine, melphalan, and thiotepa.

[b]G-CSF primed.

[c]Depends on the source of stem cells.

[d]Higher with the use of ATG in conditioning.

[e]Depends on conditioning and graft source.

[f]Trend towards lower rates with the use of TBI in conditioning.

[g]May not be fully functional.

acquisition, and donor availability for cellular therapies after transplantation. Unrelated donor graft acquisition cost is also prohibitive for limited-resource settings (~$50,000 for the umbilical cord, ~$20,000 for MUD).

Principles (Table 1)

Platform A

Cyclophosphamide (Cy) is given on days +3 and +4 after stem cell infusion (day 0) due to its unique ability to suppress alloreactive proliferating cells (mainly CD4+ T cells). Stem cells escape the Cy toxicity due to high aldehyde dehydrogenase level [9], which aid in Cy metabolism. Giving Cy a few days after day 0 allows the donor T cells to enter the cell cycle and proliferate after encountering alloantigens and wipe out the host BM, thus reducing the chances of GF [10]. Cy suppresses recipient T cells and causes intrathymic clonal deletion of alloreactive T cells, which helps prevent graft rejection. Cytotoxic sensitivity of proliferating T cells to Cy is higher than non-alloreactive, resting cells [11] like donor T regulatory (T_{reg}) and memory T (T_{mem}) cells. These cells escape Cy toxicity and contribute to anti-infective and GvL effects [12]. Peripheral tolerance mediated by donor T_{reg} is critical to the GvHD preventive benefits of PTCy [13]. Cy eliminates Natural Killer (NK) (almost entirely by day +8 [14]), naive B and T cells [15]. Cy must be given within 48–72 h after D0 to achieve maximal tolerance to minor histocompatibility antigens after alloantigen exposure [16]. The original Baltimore protocol used Cy on D + 3 [17], and the D + 4 dose was added to minimize rejection and GvHD [10]; pharmacologic GvHD prophylaxis is not started until the day following Cy to avoid blocking Cy-induced tolerance [18]. However, this belief is in question as studies incorporating calcineurin inhibitor (CNI) before Cy show improved GvHD prevention [19–22]. Adding ATG to conditioning for Haplo-HSCT with platform A have shown improved GvHD free survival rates in adults. However, this approach may result in a higher risk of severe viral infections and may affect the early GvL activity post-HSCT. The classic regimen [10] comprised RIC with a CNI and mycophenolate mofetil (MMF) from day +5 onwards. A study showed a reduction in GvHD risk by the replacement of MMF with Methotrexate [23]. However, a higher rate of severe acute GvHD

(aGvHD) is seen with the omission of CNI [24]. The optimal required duration of CNI post-HSCT in platform A is unclear.

Platform B

An ex-vivo donor graft processing using an immunomagnetic method (CliniMACS plus, Miltenyi Biotec, Bergisch Gladbach, Germany) [25] removes GvHD causing TCRαβ T cells. It retains CD34+ stem cells with committed progenitor cells, NK and TCRγδ T cells in the graft, which promote engraftment [26] and immune reconstitution. NK and TCRγδ T cells kill cancer cells in an MHC-independent manner [27], do not mediate GvHD [28] and also deplete mesenchymal stromal cells, a component of the tumor microenvironment [29]. Along with rituximab in the conditioning, CD19+ depletion in graft processing reduces the risk of EBV-induced post-transplant lymphoproliferation (PTLD). It also reduces the risk of cGvHD [30] and autoimmune illnesses [31]. For optimal efficacy of this approach, the following cell thresholds are recommended (per Kg of recipient body weight) in the processed graft: αβT cells $<1 \times 10^5$, B cells $<1 \times 10^5$ and CD34 cells $>5–10 \times 10^6$. NK and γδ cell content in the graft is usually $>1–10 \times 10^6$/kg recipient weight. With platform B, a MAC with low dose anti-T lymphocyte globulin (ATLG, Grafalon) (15 mg/kg total) and rituximab (200 mg/m^2) given on Day-1 is commonly used. ATLG is not available in North America, and an approximate equivalent dose of thymoglobulin (rabbit ATG) is 3–3.5 mg/kg (optimal rATG dosing unclear). ATLG is preferred over rATG because of a shorter half-life and the possibility of a higher ATG dose affecting γδ T or NK cells in the graft, increasing the risk of GF and infections [31]. The Bellicum trial is a modification of platform B where depleted αβT cells are genetically engineered (BPX−501) by incorporating CaspaCIDe® safety switch, based on a fusion of human caspase 9 to human FK506-binding protein, and given back to recipient around Day +14. These cells reduce viral infections and improve immune reconstitution with possibly better GvL effect. If GvHD occurs, the switch can be activated by rimiducid, and alloreactive T cells get eliminated [32]; this approach has shown promising results in children [33].

Platform C

Murine models demonstrated that the T_{naive} [CD45RA + CD62L +] T-cell subset is the leading cause of severe GvHD, and central memory T cells (T_{CM}) cause only a limited GvHD but contribute to the GvL effect [34, 35]. The hypothesis behind this observation is that the T_{naive} subset is antigen inexperienced and has a more diverse TCR repertoire and a higher frequency of minor H antigen-specific T cells than T_{mem} cells [36, 37]. Bleakley et al. developed a two-step graft processing strategy [38] to deplete CD45RA cells from the graft and retain T_{mem} cells. CD34+ stem cells (also express CD45RA) are selected from G-CSF-mobilized apheresis products, followed by depletion of CD45RA+ cells from CD34 depleted fraction using murine anti-CD45RA monoclonal antibodies. Terminally differentiated effector memory re-expressing CD45RA cells and B cells are also removed [39]. Post CD45RA depletion, the targeted T-cell content is 1×10^7 cells/Kg in the graft. The resulting naive T-cell content in the processed graft is $<5–7.5 \times 10^4$/Kg recipient weight. In addition to a 4.5–5.0-log depletion of naive T cells, CD45RA-depleted products contain a lower number of Treg, B, γδ T, and NK cells (all express CD45RA). The CD34-CD45RA- fraction from the second selection step is infused into the patient, along with the CD34+ fraction.

Platform D

G-CSF can modulate T cell tolerance through direct and indirect pathways [40] and cause T-cell polarization from Th1 to Th2 phenotype and T cell/Th17 balance toward T_{reg} cells. The Beijing group first applied this principle in the Haplo-HSCT setting by using the GIAC platform. [41] The findings; CD4:CD8 ratio ≥1.16 in BM increases the risk of aGvHD [42] and CD56^{bright} NK cell dose in the graft $>1.9 \times 10^6$/Kg increases the risk of cGvHD [43], forms the basis of risk-stratified GvHD prophylaxis in the Beijing protocol. Patients with high CD4:CD8 ratio in BM or CD56^{bright} NK cell content in the graft receives additional methylprednisolone from days 5–30 post-HSCT. [0.5 mg/kg/d on days 5–12, then tapering doses from days 13–30] [44]. This strategy is mainly studied in the adult population. The non-HLA donor selection criteria included in the Beijing protocol [8] are donor-specific antibodies, donor sex, preference to young (<30-year age), preference to Killer immunoglobulin-like receptor (KIR) ligand–ligand match or non-inherited maternal antigen (NIMA) mismatch and the use of risk-stratified DLI post-HSCT [45]. The detailed donor selection criteria can be found elsewhere [46]. The Rome Transplant Network used the GIAC concept but used bone marrow alone rather than a combination of marrow and peripheral blood cells and had strengthened GVHD prophylaxis by adding anti-CD25 antibody [47] on Day 0 and Day +4.

Drawbacks

Platform A

Cytokine release syndrome (CRS) can happen during the first-week post-HSCT [48] and often does not require

administration of steroids or tocilizumab. Severe CRS is limited to PBSC graft and associated with pre-transplant active disease, HLADRB1 mismatch [49]. Cy contributes to the risk of veno-occlusive disease (VOD), hemorrhagic cystitis (HC), and mucositis. VOD rates in children getting HSCT with PTCy vary (5–20% [50–52]). HC is a known side effect of high dose Cy [53, 54], and usually, it is mediated by the BK virus. A Colombian study reported 36% of patients developing HC in children with leukemia undergoing transplant with MAC-PTCy [23]. A prospective trial involving children and adults with leukemia showed 20% of patients developing HC (49% with grade III/IV) after receiving MAC and PTCy for HSCT [55]. There is a small risk of bladder carcinoma in patients who develop HC [56], and the Children Oncology Group recommends monitoring for the same in children with cumulative Cy exposure ≥3 g/m^2 or 100 mg/kg. Post-HSCT macrophage activation is a complication with mortality risk (incidence up to ~12%) [57, 58].

T-cell depletion

The challenges with platforms B and C are (1) need for regulatory approvals for cellular processing, (2) training of lab personnel and laboratory infrastructure (cell washing, CliniMACS device, flowcytometry support), (3) High graft processing costs. Most published studies included the CliniMACS Plus system for graft processing but, an automated cell processing closed system is now available (CliniMACS Prodigy®). It bypasses the need for robust laboratory infrastructure and manual handling steps up to some extent. There is a concern of losing a graft if Prodigy® automation is defective. Recent Danish series described its use in ten patients [59], and the depletion data were comparable to the CliniMACS Plus. TCRαβ cell depletion increases viral infection risk in the early post-HSCT period. Evidence suggests the risk may be equivalent to MUD transplants [60]. VOD is rare with platform B [60–62], partly because most studies used treosulfan instead of busulfan. The GvHD preventing benefits of naive T-cell depletion is unclear due to conflicting evidence.

The Beijing protocol

The main drawback of the Beijing protocol is a relatively higher risk of GvHD (especially chronic) (Tables 2–4) and a need for the donor to undergo two stem cell collection procedures. Also, involved donor-recipient testing requirements make it challenging to replicate in resource-poor settings. The Beijing protocol is associated with significant HC risk post-HSCT [44] [attributed to high dose Cy (3.6 g/m^2) in conditioning]. Moreover, the unfamiliarity of its use outside China also makes it a seldom used approach in the Western world. However, there are encouraging data by the Italian group using a modification of this platform.

Evidence (Tables 2, 3, 4)

The outcomes of children with hematological malignancies undergoing HSCT with any of the platforms are very promising (Tables 2–4). However, the interpretation and comparison of different approaches are difficult due to the heterogeneous and retrospective nature of studies involving a small number of patients. The potential factors affecting the outcomes of T replete Haplo-HSCT are shown in Fig. 1.

In the first report from Japan [63], 15 children (nine with leukemias, six with neuroblastoma) underwent RIC-HSCT using the PTCy platform. The outcomes were poor (11/15 progressing/relapsing) and were attributed to both refractory diseases at the time of HSCT (2 survivors were in CR2) and RIC use. Also, 25% developed severe cGvHD, which was likely related to using only a single dose of Cy on Day +3. Klein et al. reported the use of RIC conditioning in children and young adults with leukemia and showed low non-relapse mortality (NRM) but high incidences of relapses [64]. Studies have shown that relapse risk can be reduced using MAC [65–67] with platform A. An Arizona group reported outcomes of PTCY haplo with MAC [67, 68]. They showed 74% disease-free survival at 25 months median follow-up in 21 patients (15 getting PTCy and 6 PTCy +Bendamustine). In this study, four patients were not in remission at transplant, and two survived disease-free post-HSCT. All 13 patients with ALL were MRD negative before conditioning in this study, and all except one had successful transplant outcomes. A recent abstract showed a 48% incidence of GF with the use of PTCy with RIC (Flu/Cy/2 Gy TBI) in 27 children with leukemias [69]; in contrast, an Italian study using a similar regimen in 19 children showed only 1 case of GF (in a child with donor-specific HLA antibodies) [70]. This study also showed a lower relapse incidence with maternal graft [70]. A recent prospective study [55] showed that using MAC with PTCy is efficacious and has a low incidence (6%) of NRM in children. This study also showed a modest increase in the risk of relapse [HR 1.9, p 0.05] for patients in morphologic CR but with MRD positivity pre-BMT. Evidence supports the use of RIC regimens in children with leukemias with negative MRD before HSCT [71]; this remains to be tested in a Haplo-HSCT setting.

Lang et al. [72] were the first to report findings in children with leukemia undergoing MAC HSCT with platform B. Patients who received a first Haplo-HSCT in CR1-CR3 showed 100% survival at 1 year whereas no patient with

Table 2 Haplo-PTCy in children with hematological malignancies.

	No. of patients [age range in years]	Conditioning	GF% (CI/ proportion) [graft source]	aGvHD % (II–IV/ III–IV)	cGvHD % (overall/ extensive)	CR1/2 % before HSCT	NRM%	Relapse % (CI/ proportion)	DFS/OS (%)
Jaiswal et al. [51]	20 [2–20]	MAC Bu + Flu + Mel	0 [PBSC]	35/20	5/0	35	20	25.7	59/64 @ 2 years
González-Llano et al. [125]	25 [1–21]	MAC Bu + Flu + Cy	4 [PBSC]	43/19	15	44	36	40	33/50 @ 1 year 90 for those transplanted in CR1
Berger et al. [70]	33 [1–21]	RIC (57%)- Flu + Cy + TBI 2 Gy MAC (43%)- Bu + Flu + TT	3 [BM]	22/3	4/NA	54 (15% >CR2)	9	24	61/72 @ 1 year
Klein et al. [64]	40 [1–25]	RIC Flu + Cy + TBI 2 Gy	9 [BM]	33/5	23/7	60	13	52	43/56 (72% OS for <18-year-old) @ 1 year
Dufort et al. [65]	23[1–26]	MAC (70%) TBI/Bu + Cy/VP16	13 [PBSC]	45/5	53	65 (8.6 in CR3)	26	24	–/48 @17 months
Trujillo et al. [87] ASH abstract	39 [2–17]	RIC Bu/Mel + Flu + TBI 4 Gy	NA [PBSC]	25/13	–/16.6	74	15.4	23	48/51 @ 3 years [87/75 for those in CR1]
Hong et al. [50]	34 [0.9–20.3] (11 nonmalignant)	MAC Bu + Cy + Flu	3 [PBSC]	38.2/6	–/9	73 (27 in >CR2)	2.9	21.7	78.3/82 @ 2 years
Uygun et al. [6]	62 [0.4–10] (39 malignant)	MAC (89%) Bu based in majority.	6 [BM + PBSC]	47/26	21/5	28 CR1 72 ≥ CR2	NA	18	59/64.6 @ 2 years
Medina et al. [23]	52 [1.2–17]	MAC Bu + Flu + TBI (4 Gy)/Mel	NA [BM 60%]	42/8.5	19/NA	90	18	NA	57/59 @ 5 years
Perez-Martinez et al. [95]	41	MAC Bu/Mel + Flu + TT	9.8 [PBSC 78%]	–/28.2	47.7/–	64 (MRD neg)	5.4	26.8	57/65.4 @ 2 years
Symons et al. [55]	29 [1–24]	MAC BuCY (55%) CYTBI (45%)	NA [BM]	17/4	28/14	100	7	28	69/79 @ 3 years
Katsanis et al. [67]	21 [1.1–24.7]	MAC TBI + Flu or Bu + Flu + Mel	4.7 [BM]	30.3/15	18.1/12	57 (23.8 in >CR2)	9.5	17.6	74/84 @ 2 years

AML acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *BM* bone marrow, *Bu* busulfan, *CI* cumulative incidence, *CR* complete remission, *Cy* cyclophosphamide, *DFS* disease-free survival, *DLI* donor lymphocyte infusion, *Flu* fludarabine, *GF* graft failure, *MAC* myeloablative conditioning, *Mel* melphalan, *NRM* non-relapse mortality, *OS* overall survival, *PBSC* peripheral blood stem cell, *RIC* reduced-intensity conditioning, *TBI* total body irradiation, *Treo* treosulfan, *TT* thiotepa, *VP16* etoposide.

Table 3 TCRαβ-Haplo in children with hematological malignancies.

	No. of patients [age range in years]	Conditioning	GF% [CI/ Proportion]	aGvHD % (II-IV/III-IV)	cGvHD % (overall/ extensive)	CR1/2 % before HSCT	NRM%	Relapse % [CI/ Proportion]	DFS/OS (%)
Lang et al. [72]	41 [2–18] (5-Nonmalignant)	RIC Flu + Mel + TT and OKT3/ATLG	12	25/15	28/9.3	29	NA	41.4	100/NA (CR1–3) (29% for those in CR2–6) @ 1.6 years
Maschan et al. [90]	33 [1–23] (13 Haplo, 20 MUD in AML)	MAC Treo + Flu + Mel and eATG	0	39/16	30/13.3 (7/10 were on DLI)	81	10 (17% MUD, 0% Haplo)	31 (25% MUD and 40% Haplo)	68/67 @ 2 years 33% EFS for HSCT in active disease
Shelikhova et al. [79]	67 [0.15–20] (T ALL-26, B-ALL-41) 42-Haplo, 25 MUD	MAC Treo or TBI + serotherapy	1.5%	23.9/7.5	22.9/NA	80.5	17 at 2 years	32	49.6/50 @ 2 years
Lang et al. [73, 126] abstract	30 [1–17]	RIC Flu + Mel + TT and ATLG or TNI	23.3	3.3/0	9/9	13 in CR1	16	27	60/64 @ 1 year
Erbey F et al. [127]	21 [10.8-year median] (14 ALL, 7 AML)	RIC Flu + Mel + TT and rATG + Ritux + MSC	14.3%	33.3/–	19	100 (19 in CR1)	16.3	9.5	86.9/71 @ 5 years
Locatelli F et al. Blood [77]	80 [0.9–20.9] (ALL 56, AML 24)	MAC (TBI + TT + Flu/Mel) in 75%	10	NA/0	5/0 (skin only limited)	100	5	23 for ALL 28 for AML	71/72 @ 5 years
Maschan et al. [91]	73 [median 6.8 years] [37 Haplo, 36 MUD in AML]	MAC (Treo + Flu + Mel/TT and eATG ± MTX or rATG + ritux + Bortezomib	1.3%	19/6	22/7	100	10	13 (Haplo) 30 (MUD)	81/86 (Haplo) 55/64 (MUD) @ 3 years
Bertania et al. [60].	98 [0.1–17.3] (Haplo-SCT)	MAC (74% TBI based) TBI/Bu based +ATLG/ rATG +Ritux	2	16/0	6/1	100	9%	29	62/67 @ 5 years
Galaverna et al. [33] [Bellicum Trial]	100 [1.1–17.94]	MAC (TBI based)	4.1	–/3.1	18.1/3.6	100	4.8 (ALL) 8.8 (AML)	NA	80/89 (ALL) 84.7/91 (AML) Median 13–14 months f/u
Shelikhova L et al. [74]	22 [1–18] Refractory AML	MAC (Treo + Mel/TT + Ritux/ +Bortezomib Tocilizumab + Abatacept) (DLI in 17)	NA	18/ (3/4 with Gr III–IV)	23/NA	00	9	42	49/53 @ 2 years (10 received 5-azacytidine after D0)
Perez-Martinez et al. [95]	34	MAC [Bu/Mel + Flu + TT]	5.8	/14.7	15.7/	NA	14.7	28	53/59 @ 2 years

AML acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *eATG* equine anti-thymocyte globulin, *ATLG* anti-T lymphocyte globulin, *Bu* busulfan, *CI* cumulative incidence, *CR* complete remission, *Cy* cyclophosphamide, *DFS* disease-free survival, *DLI* donor lymphocyte infusion, *Flu* fludarabine, *GF* graft failure, *MAC* myeloablative conditioning, *MDS* myelodysplastic syndrome, *Mel* melphalan, *MSC* mesenchymal stem cells, *NRM* non-relapse mortality, *OS* overall survival, *RIC* reduced-intensity conditioning, *SOT* solid organ transplant, *Treo* treosulfan, *TT* thiotepa, *VP16* etoposide.

Table 4 Beijing Haplo protocol in children with Hematological Malignancies[@].

	No. of patients [age range in years]	GF% (CI/ proportion)	aGvHD % (II–IV/ III–IV)	cGvHD % (overall/ extensive)	CR1/2 % before HSCT	NRM %	Relapse % (CI/ proportion)	DFS/OS (%)
Liu et al. [82]	212 [3–18] ALL (63%) AML (37%)	0	41/14	40/27	84	19(ALL) 13(AML)	29 (ALL) 16 (AML)	57/63 (ALL) 73/73 (AML) @ 5 years
Mo et al. [113]	97 [1–18] AML	NA	56/20	60/13(Ind^1S) 70/22 (Ind^1R)	100	10.8 (Ind^1S) 5(Ind^1R)	8 (Ind^1S) 22 (Ind^1R)	81/83 (Ind^1S) 72/73 (Ind^1R)
Chang et al. [112]	149 [1–19] AML	NA	28/NA	43/NA	83 (CR1)	7	21	74/76
Chen et al. [128]	50 [4–18] Ph+ ALL	0	68/15	48/26	100	16	23	61/70 @ 3 years
Xue et al. [85]	37 [5–17] Ph+ ALL	NA	66/17	46/24	100	NA	15	77/85 @ 3 years
Xue et al. [84]	42 [2–17] ALL	0	55/12	56/23	100	9	11	81/81 @ 3 years
Zheng et al. [96]	69 [1–16]	0	35/12	35/16	80	11	16	73/75 @ 3 years
Bai L et al. [129]	19 [1–14] B-ALL with rMLL	0	37/15	54/27	100	NA	5	89/87 @ 4 years

[@]Uniform conditioning was used in Beijing protocol: cytarabine (4 g/m [2]/day from days −10 to −9); busulfan (3.2 mg/kg/d from days −8 to −6); Cy (1.8 g/m^2/d from days −5 to −4); simustine (250 mg/kg/d on day −3), and rATG (2.5 mg/kg/d from days −5 to −2).

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *CI* cumulative incidence, *CR* complete remission, *DFS* disease-free survival, *GF* graft failure, *Ind^1S* sensitive to induction 1, *Ind^1R* resistant to induction 1, *OS* overall survival, *rMLL* MLL rearrangement.

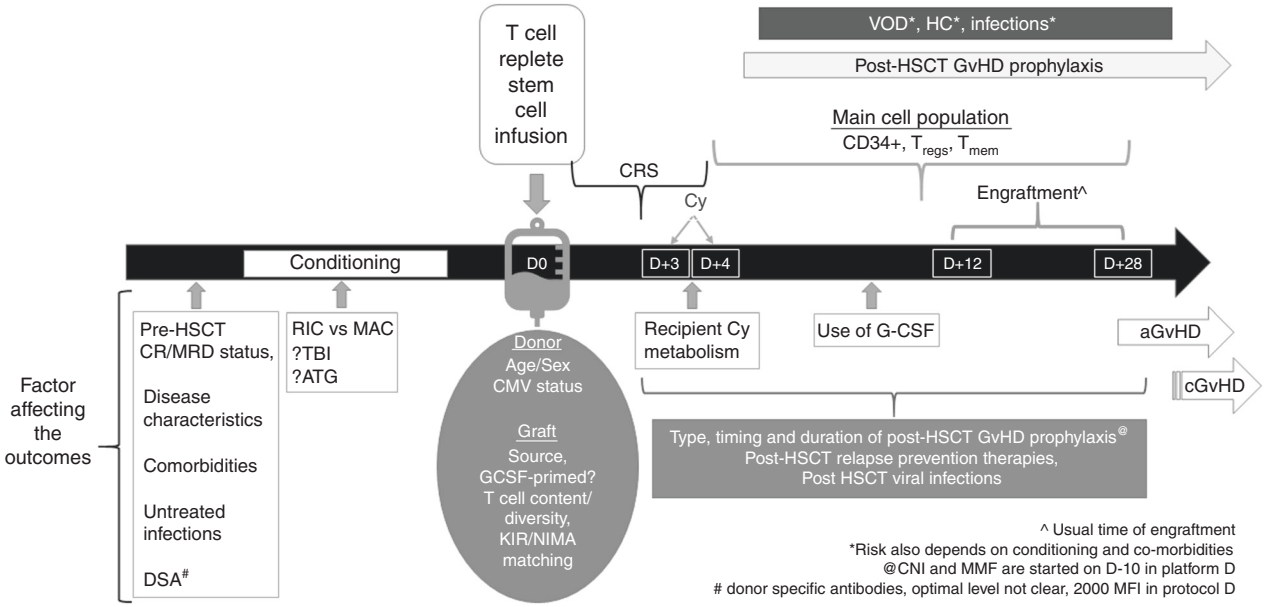

Fig. 1 T-cell replete Haplo-HSCT and factors affecting its outcomes. ATLG anti-T lymphocyte globulin, BM bone marrow, CNI calcineurin inhibitor, CRS cytokine release syndrome, Cy cyclophosphamide, G-CSF granulocyte colony-stimulating factor, HC hemorrhagic cystitis, KIR killer immunoglobulin-like receptor, MAS macrophage activation syndrome, MFI mean fluorescence intensity, NIMA non-inherited maternal antigen, PBSC peripheral blood stem cells, VOD veno-occlusive disease.

active disease survived. This group also reported the successful use of RIC with platform B in a small cohort of children from a prospective study [73]. Shelikhova et al. [74] reported a 49% EFS in those with refractory AML undergoing MAC Haplo-HSCT using modified platform B without ATG but with post-HSCT CD45RA-depleted donor lymphocyte infusion (DLI). Contrary to many other studies, this study did show promising outcomes (49% DFS) in children with refractory AML. Due to the heterogeneous nature of modifications in this study (use of DLI, the addition of costimulatory blockade, use of hypomethylating agents), it is difficult to conclude which intervention contributed to improved outcomes in children with refractory disease. Jacoby et al. [75] showed improved outcomes with intensified conditioning regimen in children with leukemia with 62% EFS. None of the ten children getting MAC (TBI based) developed GF whilst 6/8 children getting chemo based [fludarabine, melphalan, and thiotepa with ATG] conditioning developed GF [76]. Locatelli et al. [77] reported excellent disease-free survival (DFS 71%) in 80 children with leukemia undergoing transplant in remission using platform B, and the outcomes were comparable with MSD or MUD HSCT. In multivariate analysis, only the use of TBI in conditioning affected DFS, and the results remained the same in the study update presented at ASH 2018 [78]. A multicenter study [60] involving 343 leukemia patients (98 with αβHaplo) confirmed these findings, except it did not show TBI's protective effect on the relapse risk. It showed cGVHD/DFS outcomes with platform B comparable to MUD transplants and superior to MMUD transplants (61% for αβHaplo, 58% for MUD, and 34% for MMUD) [60]. A Russian study [79] showed a higher survival trend with TBI-based conditioning than treosulfan-based conditioning for platform B in children with ALL. Maschan et al. [80] presented results of αβHaplo ($n = 37$) and αβMUD ($n = 36$) transplants in children with AML and showed a lower relapse risk using haplo donor compared to MUD (9 vs 31%) with an excellent DFS in Haplo-HSCT group (86 vs 55%).

Liu et al. [81] first reported the safety and efficacy of the Beijing protocol in children with leukemia and subsequently updated promising long-term data [82] with outcomes equivalent to MSD-HSCT. A randomized trial [83] showed a favorable outcome of using Haplo-HSCT in patients with ALL and positive MRD pre-HSCT. Use of Beijing protocol in children with very high-risk B-ALL in CR1 showed superior outcomes compared to chemotherapy alone. [DFS 81 vs 52%, OS 80 vs 62%] [84]. In the subgroup analysis, Haplo-HSCT only remained beneficial for those with persistent MRD positivity or conversion of MRD from negative to positive. Similarly, patients with Ph + ALL and high-risk features (not achieving remission at the end of induction or ≥3 molecular log reduction at three months after starting therapy) benefited from Haplo-HSCT [85]. Interestingly, a large study with a prospective and retrospective cohort of children and adults with AML showed that Haplo-HSCT using platform D (with or without DLI) could nullify the effect of pre-HSCT MRD positivity on transplant outcomes in contrast to MSD-HSCT [86].

Which Platform prevents GvHD better?

Studies in adults commonly show a low incidence of severe aGvHD [10, 22] with platform A, but the data are not consistent in children, possibly because of higher alloreactivity resulting from variable Cy metabolism. A Columbian abstract [87] demonstrated a 29.4% incidence in <10-year-old versus a 0% incidence in ≥10-year-old children, and an Indian study made similar observations [51]. In contrast, a Korean PTCy study [50] did not show an age-related aGvHD difference in children. The occurrence of GvHD with platform A also depends on the graft source. Bone marrow graft is associated with a lower risk of GvHD but a higher risk of GF [88, 89]. For cGvHD, the preventive effect of PTCy may not be as good as for aGvHD with the use of PBSC graft [65]; however, it is still comparable to the MUD HSCT. Recent Pediatric Blood and Marrow transplant consortium data show a 4% rate of moderate-severe cGvHD at one year. Dufort et al. [65] showed similar aGvHD, NRM, and survival rates in children undergoing haplo transplantation with platform A or B, but cGvHD in the ex vivo T-cell depletion group was lower (9%) compared to the PTCy group (53%).

While low grade (Grade I/II) skin aGvHD is commonly reported with the use of platform B, it is rarely (<5% incidence) associated with visceral, severe skin or cGvHD [60]. A small quantity of TCRαβ cells in the processed graft are a cause for mild aGvHD [5], but its low content and a high number of γδ T cells in the graft guarantees near absence of severe aGvHD or cGvHD. Maschan et al. [90] reported a higher occurrence of cGvHD (30%) with the use of platform B in children with AML, but it was likely related to the DLI use. The same group reported a higher incidence of aGVHD and cGvHD with the horse ATG use in conditioning compared to rATG use [79, 91].

With platform C, the evidence is limited. In a small adult study [92], the frequency and pattern of aGvHD were like the T replete graft transplants, but GvHD was always responsive to steroids. A study of 17 children with high-risk hematologic malignancies undergoing HSCT with platform C [93] showed no aGvHD occurrence; but 6/17 patients developed cGvHD. Recently, St Jude investigators reported a significant rate of grade III/IV acute (28%) and chronic GvHD (26%) in 50 children undergoing CD45RA-depleted haplo HSCT as a part of an ongoing trial [94]. These data show that T_{mem} cells have the potential to cause severe GvHD as a relatively higher dose of Tmem cells was given (median 76 million/kg) compared to HLA-matched HSCT. In this study, a donor with the KIR mismatch was preferred, and donor NK cells were also infused in the recipient. A Spanish study [95] analyzing retrospective data of 192 children undergoing haploidentical transplant with PTCy or various T-cell-depleted platforms showed significantly high rates of grade I–II aGvHD [2-year probability 52.6% in PTCy vs 27.1 in TCRαβ vs 68.4 in CD45RA TCD] and cGvHD (mostly limited) in PTCy platform [2-year probability 47.7% in PTCy vs 15.7 in TCRαβ vs 13.3 in CD45RA TCD]. This study did show a lower occurrence (<10%) of severe aGvHD in Platform B compared to A (30%) or C (38%).

Overall, both acute and chronic GvHD frequencies seem higher with platform D compared to other platforms (Table 4). This is also noticed in the comparative studies with HLA-matched transplants [96]. It is explained by high T-cell content in the graft (average inoculum 1.5×10^8 T cells/Kg [97]) and the use of PBSC as a stem cell source in addition to BM in all patients. The modification of this platform by the Rome transplant network using only BM as a graft source does not show such higher incidences of GvHD in adults [98]. Wang et al. reported that adding low dose PTCy to the "Beijing Protocol" reduced GvHD and facilitated suppressive Tregs reconstitution, which might enhance the GvHD protection [99].

Engraftment, immune reconstitution, and autoimmunity

The risk of GF with novel platforms is lower than traditional haplo-transplant approaches (Tables 2–4). Immune reconstitution seems robust and is comparable to HLA-matched transplants [70]; however, comparative studies are lacking in a homogenous population. A median time to neutrophil and platelet engraftment with platform B and D is commonly reported to be earlier (10–18 days) compared to Platform A (15–30 days post-HSCT) [23, 55]. One must consider the type of conditioning and serotherapy used to interpret immune reconstitution and GF data. Besides, CMV infection can affect the pattern of immune reconstitution of both adaptive and innate cells. Effector memory and γδ T-cell subsets show an early recovery in patients undergoing PTCy based [100] and αβ T cells depleted HSCT [5], respectively and both expand in response to CMV [100]. NK cell recovery is delayed with platform A [101] compared to platform B [5], and phenotypic rescue may take 9–12 months after HSCT. Intensive GvHD prophylaxis use in platform D may suppress functional immune recovery. Triplett et al. [93] showed early T-cell recovery comprising mainly of T_{mem} and T_{reg} subsets with platform C. A retrospective Spanish study showed early NK and B cell recovery in ex vivo TCD platforms; however, CD4 and CD8 cell recovery were earlier (Day +60 and Day +90) with the PTCy platform [95]. By 6-month post-HSCT, there was no significant difference in the CD4, NK, and B cell numbers between platforms A, B, or C.

GvL effect and relapse post-HSCT

The prime mediators of the early GvL effect in different platforms are shown in Table 1. In platform A or D, a significant population of alloreactive T cells escapes the in vivo purging by Cy or ATG and are capable of mediating the GvL effect. In platform A, reconstituting NK cells, post-HSCT, have an immature phenotype [CD62L + NKG2A + KIR-] with impaired GvL effect [14]. Roberto et al. described an unconventional subset of NK cells [$p46^{neg/low}CD56^{dim}CD16^{neg}$] appearing in 2^{nd}-week post-HSCT in platform A [102]. Although retaining their proliferative capacity, this subset showed defective in vitro cytotoxicity due to high-level expression of inhibitory receptor CD94/NKG2A. An adult trial comparing double umbilical cord blood (UCB) vs PTCy-haplo showed that PTCy was associated with higher relapse rates [103]; one hypothesis was the weak NK cell-mediated GvL effect in platform A. There is also evidence that NK cells may not achieve full functionality until after 1-year post-TCRαβ depleted HSCT [104]. Enhanced cytotoxic activity of γδ T cells and improved outcomes were demonstrated in children getting aminobisphosphonates (zoledronic acid) after TCRαβHaplo [105]. While Vδ2 subset of TCRγδ cells provides the main GvL effect, Vδ1 cells are also cytotoxic against ALL, AML or CLL cells [106], and these cells expand in the presence of CMV, thus explaining the protective effect of CMV reactivation on the risk of leukemia relapse. Whether the omission of ATG from the transplant conditioning improves the GvL effect in platform B needs to be seen. Shelikhova et al. [74] tried removing ATG in children with refractory AML undergoing TCRαβHaplo and replaced it with costimulation blockade (abatacept plus tocilizumab). This study did show promising outcomes in children with refractory leukemia, but which intervention resulted in enhanced GvL effect is difficult to say because of multiple interventions and a small number of patients.

NK cells are crucial mediators of the GvL effect (mainly in myeloid disease) in Haplo-HSCT [107, 108]. Perugia group first confirmed the role of NK KIR ligand–ligand mismatch in reducing the risk of relapse in patients with AML undergoing T-cell-depleted, mega stem cell dose haploidentical transplantation [1]. However, the opposite observation made in platform B or D [109]. Theoretically, one can exploit NK alloreactivity in platform B, C, or D to the recipient advantage. However, there is conflicting evidence, whether choosing an NK alloreactive donor results in improved outcomes of these haplo-platforms [60, 70]. It is also difficult to compare studies using different models of NK alloreactivity. A Spanish study in children showed no benefit of donor–recipient KIR mismatch on outcomes for either PTCy or TCD platforms [95]. A study in adults with hematological malignancies found that KIR-ligand mismatch was associated with a lower incidence of relapse for patients undergoing PTCy-Haplo-HSCT but had no impact on those transplanted in CR [110]. In contrast, a study in adults [111] showed a detrimental effect of KIR ligand mismatching in Haplo-HSCT using PTCy. A Russian study using platform B for Haplo or MUD HSCT in children with AML in remission did not find any effect of KIR mismatch on outcomes [91]. The discordant results may reflect differences in NK recovery, interaction with T cells, KIR haplotypes or undefined confounders. Studies using platform D show superior outcomes in children with high-risk ALL and AML [112] compared to MSD-HSCT, implying a superior GvL effect. In a Chinese study, children with high-risk AML resistant to induction chemotherapy (negative prognostic factor) and undergoing Haplo-HSCT in CR1 had comparable outcomes to those with good response to induction chemotherapy [113]. An analysis from China confirmed the role of mild-moderate cGvHD in the relapse risk reduction and better survival in children with hematological malignancies [114].

Poor remission status before HSCT is the main limiting factor affecting the success of Haplo-HSCT using any of these platforms, with studies showing better survival for those in CR1/CR2 vs >CR2 or not in remission [6, 82]. However, MRD status is not uniformly reported, which makes the comparison of data difficult. Other factors affecting outcomes are discussed throughout this review and may be different for each Platform. A large multicentric Spanish study showed a higher risk of relapse in those with lymphoid malignancy, a donor KIR A haplotype and positive MRD pre-transplant for children undergoing HSCT with either PTCy or ex-vivo TCD platforms [95].

The understanding of relapse mechanisms in Haplo-HSCT is evolving [115]. Concomitant loss of unshared haplotype is one of the main mechanisms of relapse in T replete Haplo-HSCT. Some of the other relapse mechanisms are HLA class II downregulation, upregulation of T-cell inhibitory ligands on leukemic blasts and change in the microenvironment surrounding leukemia cells. Studies are needed to understand the mechanisms of relapse in different Haplo-HSCT platforms. Research into different strategies for relapse prevention like DLI, NK cell infusion, donor-derived CAR-T cell therapies, Bi-specific T-cell engagers, chemotherapy, or immunomodulatory agents will also help refine these platforms. The Beijing platform uses G-CSF mobilized DLI in patients with MRD positivity post-HSCT. A study using this approach in children and adults showed a reduced relapse rate without increased risk of severe GvHD [45]. A Chinese group showed safe use of donor-derived CD19 CAR-T in two patients after Haplo-HSCT [116] as a preventive strategy. They gave CAR-T product on day 60 and 61 and showed continued proliferation of CAR-T cells despite ongoing immune suppression with no

significant GvHD occurrence in a short follow-up. A depleted fraction of donor αβT cells in Platform B can be genome-edited to generate CAR-T product, and this approach has been successfully tested in a xenograft model [117]. Researches from St Jude hospital used NK cell infusion post CD45RA-depleted HSCT in children with leukemia [94] and did show a lower incidence of relapse. However, the contribution of NK cell infusions in relapse prevention is unclear from this study.

Infection risk post-HSCT

T cells, in general, partly retain their anti-infective properties in platforms A and D [118], but there is an increased infection risk with the intensive GvHD prophylaxis. The T-cell numbers rapidly expand post-HSCT in Platform B, but it is not associated with protective TCR diversity [119]. Viral infections are common (especially CMV) in the first 3–6 months after Haplo-HSCT with any platform. Incidence of reported CMV reactivation after platform A ranges from 38 to 76% [50, 53, 55, 64] and is comparable to incidence published with platform B [5, 90, 95] and D [44]. BK virus-associated hemorrhagic cystitis (BKHC) incidence with platform A is 0–35% [50, 52, 120], with lower frequencies found with RIC regimens. With platform B, BKHC is uncommon [5, 62, 77], probably because Cy is rarely used in conditioning. The occurrence of EBV-PTLD is rare, with any of the haplo platforms described in this review [6, 44, 55, 95]. A study assessing platform B shows that despite high viral reactivations, transplant outcomes are not affected [121] except when a patient undergoes HSCT with an active systemic viral infection [5]. In a retrospective study [60], a lower risk of bacterial infections in the TCRαβ Haplo (8%) group was found in comparison with MUD (17%) and the MMUD-HSCT (34%) groups, which is partly explained by faster engraftment of neutrophils in TCRαβ group. A study in children confirmed a lower frequency of viral infection and severity associated with naive TCD HSCT than pan TCD HSCT [122]. However, an unusually higher rate of HHV-6 encephalitis was found [123]. A recent update from the St Jude cohort shows low NRM in the naive TCD-Haplo cohort compared to other Haplo cohort, which may be related to enhanced viral infections control with this Platform [94]. A retrospective comparison of the PTCy platform vs other ex vivo TCD platforms in children with leukemias did not show any difference in the probabilities of bacterial, viral, or fungal infections between platforms [95].

Non-relapse mortality (NRM)

NRM is low using any of the novel Haplo-HSCT platforms (Tables 2–4). When assessing NRM, one must consider the patient's underlying comorbidities and CR status at the time of transplantation. For example, A study in children using Beijing protocol showed Higher NRM in patients in >CR2 staus before transplant [82].

Cost-effectiveness

Studies are lacking examining the long-term cost-effectiveness of new Haplo-HSCT platforms. PTCy is cheaper (~$100 for Cy) than platform B or C (~$13,000 for graft processing) but is associated with a higher incidence of HC, VOD, and possibly GvHD, adding to the cost of care. Besides, post-HSCT GvHD prophylaxis and its monitoring contribute to the cost (approx. $6000–8000) in platforms A and D. Similarly, control of viral infections in platform B adds to the cost.

Conclusion and future direction

Haplo-HCT using novel platforms are evolving and have an established role in the field of transplantation. The outcome of such transplants seems to edge towards "non-inferiority" status compared to HLA-matched HSCT. It is impossible to identify the best Haplo platform for a given patient situation due to the reasons described in this review; however, some trends are emerging (Table 1). Access to ex-vivo graft processing, testing for KIR and NIMA, patient ability to tolerate conditioning agents, including Cy, pre-transplant disease status, and transplant center experience are the main factors to consider while choosing a Haplo-HSCT platform. Modifications of these platforms are under investigation [124], and increasing understanding of the immunobiology of Haplo-HSCT will further refine these approaches. The advantages of using PTCy include its ease of availability and low-cost administration with no need for donor graft engineering. An exceptionally low rate of cGvHD and severe aGvHD with TCRαβHaplo makes it a compelling consideration for the young (especially the one with negative pre-HSCT MRD status).

We are moving towards a paradigm shift in transplantation where Haplo-HSCT is a first-line or a preferred option in patients with leukemias. However, more studies are needed to address the areas of research gaps highlighted here. Also, reporting Haplo-HSCT data with the factors described here will make the comparison of different approaches smooth. It will be interesting to see whether a shorter time to transplantation using Haplo-HSCT improves outcomes further. Studies are needed to understand the role of maintenance chemo or donor-derived cellular therapies post HSCT. Modified use of Haplo PTCy with platforms B, C, or D will also be interesting. TCD Haplo-HSCT represents an excellent platform for adoptive immunotherapy

(NK, CAR-T) because minimal or no post-HSCT immunosuppression required. Studies are needed to confirm these platforms' efficacy compared to UCB transplants, as many physicians prefer UCB graft over Haplo-HSCT for leukemias. A comparative analysis of cost-effectiveness and quality of life outcomes is needed if the survival outcomes are comparable with different Haplo-HSCT platforms.

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Compliance with ethical standards

Conflict of interest The author declares no competing interests.

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