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Allogenic Stem Cell Transplantation from Alfabeta T/Cd19 Depleted Haploidentical Donor as Salvage Therapy in In Heavily Pretreated Relapsed/Refractory Patients with Hodgkin Lymphoma

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only potentially curative option for patients with relapsed/refractory (R/R) Hodgkin lymphoma (HL) who fail to achieve complete remission with chemotherapy, radiotherapy, or immunotherapy. However, its application has historically been limited by considerable transplant-related mortality (TRM), driven primarily by graft-versus-host disease (GVHD), infectious complications, and organ toxicity. These concerns have shaped the evolving role of allo-HSCT in HL, particularly as the integration of checkpoint inhibitors has allowed many patients to defer transplantation. Nevertheless, for heavily pretreated patients who exhaust available therapies, allo-HSCT remains indispensable, and the central challenge is to identify transplant platforms that minimize toxicity while preserving efficacy.

Reduced-intensity conditioning (RIC) regimens have improved TRM in this population, and growing evidence supports superior overall survival (OS) and progression-free survival (PFS) with RIC-based haploidentical transplantation using post-transplant cyclophosphamide (PTCy). An alternative strategy to mitigate GVHD is the *ex vivo* depletion of αβ T cells from the graft, which directly removes alloreactive T-cell populations while preserving γδ T cells and natural killer cells that may contribute to graft-versus-lymphoma activity. This approach was pioneered in pediatric patients with malignant and nonmalignant diseases and has since been consistently extended to adult hematological malignancies by our group.

Here we report the long-term outcomes of a single-center institution cohort of heavily pretreated R/R HL patients who underwent myeloablative conditioning followed by αβ T-cell/CD19-depleted haploidentical allo-HSCT. At a median follow-up of 7.5 years (range 4 months–9.5 years), no patient experienced disease relapse; two deaths without relapse occurred and are reflected in overall survival (OS, 80%) and Transplant Related Mortality (TRM). One patient died from recurrent CMV reactivation in the setting of unfavorable donor/recipient serology (in the pre-letermovir era), and one from GVHD occurring after donor lymphocyte infusion. No long-term life-threatening complications were observed in surviving patients.

These results suggest that myeloablative allo-HSCT with αβ T-cell/CD19 depletion is both effective and safe in heavily pretreated R/R HL. With the advent of letermovir, TRM is particularly low, which addresses the principal infectious cause of mortality observed in this cohort, and durable disease control is achievable even in patients with chemorefractory disease. While these data are encouraging, this platform warrants further investigation in larger, prospective studies to define its role within the current HL treatment algorithm.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (allo-HCT) remains the only potentially curative option for patients with relapsed/refractory (R/R) classical Hodgkin lymphoma (cHL) who fail to achieve durable remission with salvage chemotherapy, autologous transplantation, and novel agents [1,2]. Approximately 10–20% of cHL patients develop relapsed or refractory disease [1], and while approximately 50% of fit patients achieve durable remissions with platinum-based salvage followed by high-dose chemotherapy and autologous HCT [3,4], treatment options remain limited for those who relapse after autologous transplantation or present with highly refractory disease [5]. The introduction of brentuximab vedotin (BV) and PD-1 checkpoint inhibitors (nivolumab, pembrolizumab) has transformed the therapeutic landscape, with BV-nivolumab achieving objective response rates of 85% and complete response rates of 67% as first salvage therapy [6]. Despite these advances, responses to novel agents as monotherapy are frequently not durable, with median progression-free survival typically ranging from 9–14 months [7–9], underscoring the continued need for allo-HCT as a definitive consolidative strategy in this population [10].

However, the application of allo-HCT in cHL has historically been hampered by prohibitive transplant-related mortality (TRM). Early experiences with myeloablative conditioning (MAC) were associated with TRM rates ranging from 20% to 60%, driven primarily by graft-versus-host disease (GVHD), infectious complications, and organ toxicity [11,12]. This led to the widespread adoption of reduced-intensity conditioning (RIC), which significantly reduced nonrelapse mortality (NRM) while maintaining a graft-versus-Hodgkin-lymphoma (GvHL) effect [12]. In a subsequent EBMT analysis of 312 patients transplanted between 2006 and 2010, NRM was no longer significantly different between MAC and RIC, though MAC showed a trend toward lower relapse rates (41% vs 52% at 24 months), suggesting that modern transplant practices have narrowed the toxicity gap [13]. Contemporary single-center data confirm this evolution: in a time-trend analysis, 4-year overall survival improved from 34% in 2001–2010 to 83% in 2011–2017, with 1-year NRM declining from 34% to 5% [14]. Nevertheless, disease relapse remains the primary cause of treatment failure after RIC allo-HCT, with progression-free survival rates at 2–4 years ranging from 20–49% [15,16].

The integration of checkpoint inhibitors as bridge therapy to allo-HCT represents a critical advance but introduces new challenges. Pre-transplant PD-1 blockade improves disease control and may enhance post-transplant outcomes: in a Johns Hopkins cohort, patients receiving checkpoint inhibitors before allo-HCT with post-transplant cyclophosphamide (PTCy) achieved a 5-year overall survival of 91% versus 66% for those receiving chemotherapy alone [17]. However, prior checkpoint inhibitor exposure has been associated with increased risk and severity of acute GVHD, with grade 3–4 acute GVHD rates of 15–20% when PD-1 inhibitors are administered in the peri-transplant period [17,18]. A large joint CIBMTR/EBMT analysis of 2,186 patients confirmed that prior checkpoint inhibitor exposure increased grade II–IV acute GVHD (HR 1.26, p = 0.02), though PTCy-based GVHD prophylaxis mitigated this risk and improved overall survival [19]. These findings highlight the tension between optimizing pre-transplant disease control with immunotherapy and managing the heightened alloreactivity it generates, a tension that demands innovative GVHD prophylaxis strategies.

The donor landscape has expanded significantly with haploidentical transplantation using PTCy, which offers immediate donor availability and favorable GVHD profiles. In a landmark EBMT registry study of 709 patients, haploidentical transplantation with PTCy demonstrated comparable overall survival (67% at 2 years) and progression-free survival (43%) to matched sibling and unrelated donor transplants, with significantly lower chronic GVHD rates (26% vs 41% for matched unrelated donors) [20]. A CIBMTR analysis of 987 lymphoma patients confirmed similar survival outcomes between haploidentical and matched

sibling donor transplants, with markedly lower chronic GVHD after haploidentical transplantation (12% vs 45%, $p < 0.001$) [21]. However, even with PTCy, TRM and GVHD remain clinically significant concerns, with NRM rates of 13–17% and cumulative acute GVHD rates of 25–27% across contemporary series [20,21].

These challenges, GVHD risk (especially after checkpoint inhibitors), prolonged immunosuppression with infections, and the balance between relapse control and toxicity, highlight unmet needs targeted by alternative graft engineering strategies. In the context of haploidentical transplantation, *ex vivo* αβ T-cell depletion offers a fundamentally different approach to GVHD prevention. By selectively removing αβ T cells, the primary mediators of alloreactive GVHD, from the graft while preserving γδ T cells, natural killer (NK) cells, monocytes, and dendritic cells, this strategy directly eliminates the cellular substrate of GVHD and obviates the need for post-transplant pharmacological immunosuppression [22,23]. The retained γδ T cells and NK cells contribute to both anti-infectious immunity and graft-versus-lymphoma activity, potentially addressing the dual challenge of TRM from infections and disease relapse [22,24]. This approach was pioneered in pediatric patients with malignant and nonmalignant diseases undergoing haploidentical transplantation [25,26], and has since been extended to adult hematological malignancies, including by our group [27], with over ten years of experience. The experience with αβ T-cell depletion in cHL, however, remains scarce.

Therefore, based on this innovative transplant platform with its potential for low incidence of TRM and GVHD, since 2012 we have treated a cohort of 11 heavily pretreated patients with R/R Hodgkin lymphoma who lacked a matched related donor or who had already received a transplant from another allogeneic donor.

METHODS

Patients' accrual

Clinical records of patients with R/R HL were obtained under an approved protocol at the Hematology and Bone Marrow Transplantation Unit at the University Hospital of Parma (128/2019/OSS/AOUPR), according to the Declaration of Helsinki guidelines for the protection of human rights.

Patients and Donors

Between 2012 and 2025, 11 adult patients with R/R classical Hodgkin lymphoma were enrolled in this single-center study. In this cohort, nodular sclerosis was the predominant subtype of Hodgkin lymphoma (9/11, ~ 82%), while lymphocyte depletion and mixed cellularity were each observed in 1/11 cases (~ 9% each). One patient was lost to follow-up after discharge and was excluded from the analysis, which was therefore performed on the remaining 10 patients.

The median age at transplantation was 26 years (range 20–36). Most patients had advanced-stage disease (82% stage III–IV), and extranodal involvement was observed in 36% of cases at diagnosis. The median number of prior treatment lines was 5 (range 3–9), reflecting a heavily pretreated population. All patients except one had previously undergone autologous transplantation, and one patient had also received a prior allogeneic transplant from a matched unrelated donor (MUD). Eight patients (82%) had active disease at the time of transplant. As expected, given the young age of the study population, the Hematopoietic Cell Transplantation–Comorbidity Index (HCT-CI) was low (0–1). Patient characteristics and transplant details are summarized in Tables 1 and 2, respectively.

Table 1
Patient characteristics

Age (Y)	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**
	28	**28**	**27**	**20**	**26**	**39**	**23**	**26**	**36**
HL type	NODULAR SCLEROSIS	LYMPHOCYTE DEPLETION	NODULAR SCLEROSIS	NODULAR SCLEROSIS	NODULAR SCLEROSIS	NODULAR SCLEROSIS	NODULAR SCLEROSIS	MIXED CELLULARITY	NODULAR SCLEROSIS
BM involvement	POS	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Organ involvement	NODAL	NODAL	NODAL	NODAL	NODAL AND LUNG	NODAL	NODAL, BONE, LUNG	NODAL, LUNG, SPLEEN	NODAL
Ann Arbor stage	IV B	IV B	II A	III A	II B	IV B	III A	IV	IV B
Diagnosis date	May 2007	Jun 2013	Jul 2012	Jun 2012	Mar 2013	Jan 2010	Jul 2014	May 2017	Nov 2014
Number of lines of therapy	4	4	6	9	9	8	5	5	8
Previous therapies	ABVD	ABVD + RT	ABVD + RT	OEPA	ABVD	ABVD	ABVD	ABVD	ABVD + RT
	IEV	IGEV	IGEV	DVCP	BV	IGEV	IGEV	BEACOPP	IGEV
	BEAM + ABMT	ABMT	ABMT	COP + RT	BEACOPP	ABMT	ABMT	EDX	BV
	DAUNO LIPO	BV	BV	ABVD + RT	Benda	BV	BV	BV	NIVO
			ANTI-PD1	IEP	DHAP	BENDA	NIVO	ABMT	BV-Benda
			DHAP	DHAP	ABMT	BEACOPP			ABMT
				Dauno Lipo	NIVO	HSCT			Pembro
				ABMT	Dauno Lipo	Doxo Lipo			BV-Benda
				BV	BV-Benda	Lena			
						Dauno Lipo			
Previous RT	NO	YES	YES	YES	NO	NO	NO	NO	NO
Previous ABMT	YES	YES	YES	YES	YES	YES	YES	YES	YES

Age (Y)	#1	#2	#3	#4	#5	#6	#7	#8	#9
	28	28	27	20	26	39	23	26	36

Abbreviations:
ABMT: autologous bone marrow transplantation

ABVD: adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine

BEACOPP: cyclophosphamide, vincristine, procarbazine, and prednisone

BeGEV: bendamustine, vinorelbine, gemcitabine, and prednisolone

Benda: bendamustine

BV: brentuximab vedotin

COP: cyclophosphamide, vincristine, and prednisolone

Dauno Lipo: liposomal daunorubicin

DCP: daunorubicin, cytosine arabinoside, vincristine, and prednisolone

DHAOX: dexamethasone, high-dose cytarabine, and oxaliplatin

DHAP: dexamethasone, high-dose cytarabine, and cisplatin

Doxo Lipo: liposomal doxorubicin

EDX: cyclophosphamide

IEP: ifosfamide, epirubicin, and cisplatin

IGEV: ifosfamide, vinorelbine, gemcitabine, and prednisolone

Lena: lenalidomide

NIVO: nivolumab

OEPA: vincristine, etoposide, prednisolone, and doxorubicin

Pembro: Pembrolizumab

RT: radiotherapy

Table 2
Characteristics of allogenic transplants

Status at HSCT	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#1
	NOT CR	CR	NOT CR	NOT CR	NOT CR	NOT CR	NOT CR	CR	NOT CR	NOT CR	N
HCT-CI	0	0	0	1	0	1	0	0	0	0	0
HSCT	Mar 2013	Jun 2016	Aug 2018	Sep 2016	Nov 2016	Dec 2017	Dec 2017	Aug 2018	Feb 2019	Sep 2023	Se
CMV status R/D	POS/NEG	POS/POS	POS/NEG	POS/POS	POS/POS	NEG/POS	NEG/NEG	POS/POS	NEG/POS	POS/POS	NI
Early Complications		Recurrent Fever	Fever; mucositis	Fever; atrial flutter; pneumonia	Fever; mucositis	Fever; sepsis	Fever; TPSV	Fever	Neutropenic colitis	Fever	Fe
Rituximab for prevention of EBV reactivation			YES			YES					
Engraftment	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	ye
Days to neutrophils recovery	+9	+ 14	+15	+10	+ 12	+ 16	+11	+15	+14	+14	+1
Days to platelets recovery	+9	+11	+ 7	+13	+11	+16	+9	+10	+8	+15	+
VOD	No	no	No	no	No	no	no	no	no	no	no
MOF	no	no	no	no	No	yes	no	no	no	no	N
Day at discharge	+ 18	+ 23	+ 36	+ 31	+ 18	+ 23	+ 16	+ 13	+ 22	+ 30	+
Complications	NA	-	Fever; TMA	IRIS	-	-	-	-	Recurrent Fever, cough, congestion, upper respiratory tract infection, pneumonia	-	-
DLI	LTFU	no	no	no	No	+ 280 (total: 8 DLI)	no	no	+ 100 CD45RA	no	no
Acute GVHD	NA	-	Skin	-	-	-	-	-	+ 37 post DLI intestinal	Skin	-
Chronic GVHD	NA	-	-	-	-	-	-	-	-	-	-

Status at HSCT	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#1
	NOT CR	CR	NOT CR	NOT CR	NOT CR	NOT CR	NOT CR	CR	NOT CR	NOT CR	N(
Abbreviations:											
Status at HSCT: disease status at hematopoietic stem cell transplantation											
HCT-CI: hematopoietic cell transplantation–comorbidity index											
HSCT: hematopoietic stem cell transplantation											
CMV status R/D: cytomegalovirus serostatus (recipient/donor)											
Rituximab prophylaxis for EBV reactivation											
Engraftment: successful establishment of donor hematopoiesis											
Neu: neutrophils											
Plt: platelets											
VOD: veno-occlusive disease											
MOF: multi-organ failure											
Discharge: hospital discharge											
TMA: Thrombotic microangiopathy											
TPSV: Paroxysmal supraventricular tachycardia											
IRIS: Immune Reconstitution Inflammatory Syndrome											
Complications: post-transplant complications											
DLI: donor lymphocyte infusion											
Acute GVHD: acute graft-versus-host disease											
Chronic GVHD: chronic graft-versus-host disease											
LTFU: lost to follow up											

All donors were haploidentical family members, mismatched with the recipient at 6 of 12 HLA alleles. Donors included 6 brothers, 1 sister, 3 mothers, and 1 uncle. The median donor age was 39 years (range 20–62). Donor–recipient ABO compatibility was heterogeneous, with 6/11 matched pairs (55%) and 5/11 mismatched pairs (45%).

Pre-transplant cytomegalovirus (CMV) serology was as follows: the most unfavorable combination (donor-negative/recipient-positive) was documented in 2 cases; both donor and recipient were seropositive in 5 cases; the donor-positive/recipient-negative combination was present in 2 cases; and the most favorable combination (both seronegative) was observed in only 2 donor–recipient pairs.

Patients were selected for haploidentical transplantation because no suitable matched unrelated donor was available or because clinical urgency precluded the time required for an unrelated donor search. Written informed consent was obtained from all patients and donors. Outcomes are reported as of January 2026.

Graft Processing and Transplantation Procedures

Donors received granulocyte-colony stimulating factor (G-CSF) at 10 μg/kg body weight in two divided doses for 4–5 days to induce peripheral mobilization of hematopoietic progenitors. In one case, plerixafor was added due to insufficient mobilization. Apheresis was performed on day 5 after the start of mobilization, with a target dose of ≥ 6×10^6 $CD34^+$ cells/kg recipient body weight. G-CSF-mobilized peripheral blood progenitor cells (PBPCs) underwent αβ $TCR/CD19^+$ depletion using the CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometric analysis was performed before and after depletion to quantify $CD34^+$, $CD3^+$, αβ T cells, γδ T cells, $CD20^+$ B cells, and NK cells.

Conditioning Regimen

A chemotherapy-based myeloablative conditioning regimen was adopted to minimize extra-hematological toxicity. The regimen consisted of rabbit antithymocyte globulin (ATG, Thymoglobuline; total dose 6 mg/kg) on days − 9 to − 6, treosulfan (total dose 36 g/m²) on days − 9 to − 7, fludarabine (total dose 150 mg/m²) on days − 6 to − 2, and thiotepa (total dose 10 mg/kg) on days − 5 and − 4.

Post-Transplant Supportive Care

No pharmacological immunosuppression was administered after transplantation as GVHD prophylaxis. G-CSF was not given post-transplantation to accelerate neutrophil recovery.

In the pre-letermovir era, ganciclovir was administered during the conditioning regimen in CMV-seropositive patients; from two days before graft infusion, ganciclovir was replaced by acyclovir at 10 mg/kg. CMV reactivation was subsequently monitored by quantitative PCR and treated according to a conventional preemptive strategy. Since 2021, letermovir prophylaxis was administered according to guidelines in combination with acyclovir; only one patient in this cohort received letermovir-based prophylaxis.

Mold-active antifungal prophylaxis consisted of liposomal amphotericin B during the neutropenic phase, followed by itraconazole or posaconazole oral solution in patients at risk of invasive fungal infection, and was continued until a $CD4^+$ T-cell count of ≥ 200 cells/μL was achieved.

Monitoring and Endpoints

Chimerism analysis on peripheral blood was performed at 1-month post-transplantation, then every 2 months for 6 months, and subsequently every 4 months. Immune reconstitution was evaluated by quantification of TCR $\alpha\beta^+$ $CD3^+$, TCR $\gamma\delta^+$ $CD3^+$, $CD4^+$, $CD8^+$, NK, and $CD19^+$ cells monthly for the first 12 months after transplantation.

Disease surveillance was performed by serial PET/CT evaluations during follow-up at 3, 6 and 12 months after transplant and then every 9–12 months if negative.

Given the limited sample size, formal comparative statistical analyses were not performed; however, survival outcomes were reanalyzed using standard time-to-event definitions. Overall survival (OS) was defined as the time from HSCT to death from any cause or last follow-up. Progression-free survival (PFS) was defined as the time from HSCT to relapse, progression, death, or last follow-up, whereas relapse-free survival (RFS) was defined as the time from HSCT to relapse/progression or last disease assessment. Follow-up was estimated using the reverse Kaplan–Meier method.

RESULTS

Graft Composition

After αβ TCR/$CD19^+$ depletion, grafts contained a median of 10×10^6 $CD34^+$ cells/kg (range 6–17.5), 9×10^6 $CD3^+$ T cells/kg (range 2–20), 4.2×10^4 $\alpha\beta^+$ T cells/kg (range 1.2–7.6), 9×10^6 $\gamma\delta^+$ T cells/kg (range 2–20), 3×10^4 $CD19^+$ B cells/kg (range 1.2–6), and 22×10^6 $CD56^+$ NK cells/kg (range 1–48). Median T-cell and B-cell depletion was 4.5 and 3.2 log, respectively.

Engraftment

Full donor chimerism with sustained engraftment was achieved in all patients (100%), with no cases of graft failure. Neutrophil and platelet recovery occurred at a median of 12 days (range 9–16) and 11 days (range 8–16), respectively.

Graft-Versus-Host Disease

Acute GVHD (aGVHD) occurred in 2 of 10 evaluable patients (patients #3 and #9), both in the context of additional immunological triggers. Importantly, all cases of clinically significant acute GVHD occurred in the presence of identifiable immunological triggers rather than spontaneously.

Patient #3 experienced multiple CMV reactivations in the setting of an unfavorable serological combination (donor-negative/recipient-positive), which likely triggered cutaneous aGVHD. Treatment included corticosteroids (0.5–1 mg/kg) followed by the anti-CD30 antibody-drug conjugate brentuximab vedotin, with the rationale of discontinuing immunosuppression to foster immunological control of CMV infection. Despite these measures, the persistent inflammatory state associated with recurrent CMV reactivation ultimately led to transplant-associated thrombotic microangiopathy (TA-TMA), resulting in lower-extremity ischemia and multiorgan failure. The patient died of transplant-related complications.

Patient #9 received CD45RA-depleted donor lymphocyte infusion (DLI) at day + 100 post-transplant for recurrent upper respiratory tract infections and insufficient $CD4^+$ T-cell reconstitution. Subsequently, intestinal aGVHD developed, manifesting as diarrhea and significant weight loss. Treatment included corticosteroids and the anti-CD26 monoclonal antibody begelomab. Despite GVHD management, pulmonary infections persisted, and the patient died of respiratory failure.

No other clinically significant GVHD was observed in the remaining patients. Transient, self-limited cutaneous rash not requiring treatment was noted in isolated cases. No spontaneous GVHD attributable to the graft itself was observed.

Immune Reconstitution

Immune reconstitution kinetics are shown in Fig. 1. Following engraftment, peripheral blood T- and B-cell populations increased steadily over the first 6 months. The earliest subsets to recover were γδ T cells and NK cells, consistent with their preservation in the αβ-depleted graft. At baseline (day 30), mean ± SD values were 32.63 ± 37.52 for CD4, 40.70 ± 71.35 for CD8, 250.53 ± 238.17 for NK, 75.38 ± 85.07 for γδ T cells, and 41.60 ± 60.25 for αβ T cells. Peak group-level recovery occurred at day 180 for CD4 (231.58 ± 256.85) and γδ T cells (237.53 ± 234.21), at day 90 for NK cells (556.75 ± 922.82), and at day 350 for CD8 (297.06 ± 243.06) and αβ T cells (525.13 ± 321.26). Notably, bone marrow involvement at diagnosis did not significantly influence the kinetics or magnitude of immune reconstitution across the analyzed populations (Fig. 2).

Donor lymphocyte infusions (DLI) were administered in two patients to enhance anti-infectious immunity. $CD4^+$ T cells reached a median threshold of 200 cells/μL at approximately 5 months post-transplant, consistent with the heavily pretreated status of this cohort. This delayed adaptive immune recovery contrasted with the earlier expansion of innate-like populations, with NK and γδ T cells peaking at approximately day 90 and day 180, respectively.

Serum immunoglobulin levels showed progressive humoral immune reconstitution during the first year after transplantation. At day 30, mean ± SD values were 695.1 ± 181.3 mg/dL for IgG, 109.3 ± 88.9 mg/dL for IgA, and 27.0 ± 17.0 mg/dL for IgM. Peak group-level values were observed at day 240 for IgG (1061.3 ± 813.5 mg/dL), at day 120 for IgA (169.3 ± 99.6 mg/dL), and at day 120 for IgM (176.2 ± 195.5 mg/dL), followed by stabilization or decline by day 360. Overall, humoral recovery paralleled cellular immune reconstitution, with substantial patient-level variability.

In one patient (#9), DLI was followed by acute GVHD and severe infectious complications, suggesting a potential role of DLI in triggering immune-mediated toxicity. This patient also exhibited marked early expansion of innate immune populations, supporting a possible association between rapid immune activation and subsequent inflammatory complications. Although limited by sample size, these observations suggest a link between early innate immune expansion, delayed $CD4^+$ recovery, and the risk of post-transplant immune dysregulation.

Incidence of Infections

The rapid engraftment observed in this cohort resulted in a short period of post-conditioning aplasia, thereby reducing the risk of severe bacterial infections. Only one patient (#6/10) experienced septic shock at the onset of the conditioning regimen, which resolved with broad-spectrum antibiotics without residual organ damage. Overall, bacterial complications were infrequent and predominantly mild or localized, consistent with the rapid hematopoietic recovery. Fever represented the most common early clinical manifestation.

Viral infections were the most frequent complications, occurring in most patients across both pre- and post-engraftment periods. In the pre-engraftment phase, respiratory viruses—including rhinovirus, parainfluenza, influenza, and coronavirus, were the most detected pathogens, often identified by pharyngeal swab and generally associated with mild clinical courses. BK virus viruria was also observed in multiple patients early after conditioning.

In the early post-engraftment phase (up to day + 90), CMV reactivation occurred in 3 patients (3/10), all of whom had not received letermovir prophylaxis (transplanted before 2021), highlighting the impact of antiviral prophylaxis in this setting. All CMV episodes resolved with preemptive antiviral therapy. Beyond day + 90, viral reactivations were predominantly represented by EBV, occurring in 4 patients (4/10), often requiring rituximab to control viral replication and associated lymphadenopathy. No cases of post-transplant lymphoproliferative disease (PTLD) were observed.

Late infectious complications (beyond day + 120) included recurrent respiratory viral infections and occasional bacterial or fungal colonization, reflecting ongoing immune reconstitution. Notably, one patient (#9) developed a severe and ultimately fatal respiratory infection more than 6 months after transplantation in the context of GVHD following donor lymphocyte infusion, suggesting a potential interaction between immune dysregulation and susceptibility to infectious complications.

Overall, the temporal distribution of infections (summarized in Table 3), with early predominance of innate-controlled pathogens and later viral reactivations such as EBV, parallels the kinetics of immune reconstitution observed in this cohort, characterized by early recovery of innate immune subsets and delayed adaptive immune reconstitution

Table 3
Incidence and timing of infections (Patient #1 lost to follow-up).

PRE ENGRAFTMENT	#1	#2	#3	#4	#5	#6	#7	#8
		Bk Virus (urine)	**E.Coli (ph swab)**	**BK virus (urine)**	**Rhinovirus (ph swab)**	**Parainfluenzae (ph swab)**		
+ 40		TNF : S.Epidermidis MRSA CMV (blood)	CMV (blood)		CMV; BK virus	Parainfluenzae, Influenza A (ph swab)		Candida.Albicans + HSV1 (ph swab)
+ 60				CMV (blood)	Influenza A (ph swab)		Rhinovirus (ph swab)	
+ 90							EBV (blood)	
+ 120			CMV (blood)				HHV6 (blood)	
+ 135	Rhinovirus, MRSA (ph swab)							Candida.Albicans, Enterovirus (ph swab)
+ 150	EBV (blood)			Staphilococcus.Aureus (ph swab) CMV (blood)				
+ 180		EBV (blood)		Rhinovirus, Influenza B (ph swab) Parvovirus (BM blood)				EBV (blood)
+ 210								
+ 240								Enterovirus and candida (ph swab)
+ 270					EBV (blood)			
+ 300								
+ 365					Coronavirus (ph swab)			
Abbreviations:								
BM: bone marrow								
Ph swab: pharyngeal swab								
Cmv: cytomegalovirus								
EBV: epsteinbarr virus								
MRSA: methicillin-resistant staphylococcus aureus								

Outcome

Given the limited sample size, formal statistical analyses were not performed; however, descriptive evaluation identified clinically relevant patterns.

At a reverse Kaplan–Meier median follow-up of 7.5 years (range 4 months–9.5 years), no relapse or progression was observed. Accordingly, relapse-free survival was 100%, and the cumulative incidence of relapse was 0%. When death was included as an event, progression-free survival was 78.8%, mirroring overall survival, which was also 78.8%. Kaplan–Meier curves for OS, PFS, and RFS with 95% confidence intervals and numbers at risk are shown in Fig. 3. Notably, this encouraging outcome includes patients transplanted with active disease, suggesting that disease status at transplantation did not adversely impact relapse risk in this cohort. Eight of 10 evaluable patients remain alive. Two patients died of transplant-related complications: patient #3 due to

transplant-associated thrombotic microangiopathy in the context of recurrent CMV reactivation, and patient #9 due to pulmonary infection in the setting of GVHD following donor lymphocyte infusion, compounded by pre-existing lung disease. In this single-center institution cohort the Kaplan–Meier estimated overall survival was 78.8%, with a transplant-related mortality of 20%.

CONCLUSIONS

Allogeneic transplantation remains the only potentially curative option for patients with relapsed/refractory classical Hodgkin lymphoma who fail to achieve durable remission after multiple lines of therapy, including autologous transplantation, brentuximab vedotin, and checkpoint inhibitors [28]. However, the clinical application of allo-HCT in this setting continues to be limited by transplant-related mortality and GVHD, which remain significant even with contemporary approaches. In the PTCy-based haploidentical setting, currently the most widely adopted platform, 2-year non-relapse mortality ranges from 13% to 18%, cumulative acute GVHD rates reach 25–34%, and chronic GVHD occurs in 16–26% of patients [18,20]. Importantly, disease relapse remains the primary cause of treatment failure, with 2-year relapse rates of 24–39% across contemporary haploidentical series [18,20]. These outcomes are further complicated in the checkpoint inhibitor era, as prior PD-1 blockade has been associated with increased severity of acute GVHD after allo-HCT, with grade II–IV acute GVHD increased (HR 1.26) and grade III–IV acute GVHD increased (HR 1.41) in a joint CIBMTR/EBMT analysis of 2186 patients [19]. In a separate cohort, the 12-month cumulative incidence of grade 3–4 GVHD was 20% in patients who received checkpoint inhibitors before alloBMT with PTCy [17].

Anti-CD30 CAR T-cell therapy has emerged as an alternative cellular therapy for R/R cHL, with overall response rates of 57–72% and complete response rates of 34–59% in heavily pretreated populations [11,13]. However, responses are frequently not durable, with 1-year progression-free survival of only 36–39%, and no long-term curative plateau has been established [29,30]. CAR T-cell therapy for Hodgkin lymphoma remains investigational, with no FDA-approved anti-CD30 CAR T product currently available, and the approach is limited by the unique tumor microenvironment of cHL, in which malignant Reed-Sternberg cells represent a minority of the tumor mass [1]. Furthermore, patients who relapse after CAR T-cell therapy often still require allo-HCT as definitive consolidation, underscoring the complementary rather than competitive relationship between these cellular therapeutic modalities [31].

In this context, the present study demonstrates that haploidentical allo-HCT with *ex vivo* αβ TCR/CD19 depletion and myeloablative conditioning offers a distinct therapeutic profile that addresses several of the key limitations of both PTCy-based transplantation and CAR T-cell therapy. The most striking finding is the absence of relapse (cumulative incidence 0%) at a median follow-up of 7.5 years despite 8 of 10 patients having active disease at the time of transplant. This compares favorably with the 2-year PFS of 43–63% reported in PTCy-based haploidentical series and the 1-year PFS of 36–39% with anti-CD30 CAR T cells [29,30]. The absence of relapse in this heavily pretreated cohort, even in patients transplanted with active disease, suggests a potent graft-versus-Hodgkin-lymphoma effect mediated by the preserved γδ T cells and NK cells in the αβ-depleted graft, combined with the disease control provided by myeloablative conditioning [32,33].

The GVHD profile was notably favorable: clinically significant acute GVHD occurred in only 2 of 10 patients (20%), and both cases were triggered by specific immunological events (CMV reactivation and DLI infusion) rather than by primary alloreactivity of the graft. No spontaneous GVHD attributable to the transplant itself was observed, and no post-transplant pharmacological immunosuppression was required. This contrasts with PTCy-based haploidentical transplantation, where grade II–IV acute GVHD rates of 25–34% and chronic GVHD rates of 16–26% are reported despite prolonged immunosuppressive prophylaxis [18,20]. The elimination of post-transplant immunosuppression is a fundamental advantage of the αβ T-cell depletion platform, as it avoids the infectious complications and delayed immune reconstitution associated with calcineurin inhibitors and mycophenolate.

Notably, the two patients who received nivolumab as bridge therapy to transplant did not develop any signs of GVHD after allo-HCT, likely due to the absence of alloreactive αβ T cells in the graft. This observation is particularly relevant given the well-documented concern that prior checkpoint inhibitor exposure increases GVHD risk after conventional allo-HCT. The αβ T-cell depletion approach may therefore represent an ideal transplant platform for patients bridged with checkpoint inhibitors, effectively decoupling the benefit of pre-transplant immunotherapy from its post-transplant GVHD risk.

The overall survival of 80% reflects a TRM of 20%, with both deaths attributable to specific and potentially preventable complications. One death resulted from recurrent CMV reactivation in an unfavorable serological combination in the pre-letermovir era; the availability of letermovir prophylaxis since 2021 is expected to substantially mitigate this risk [34]. The second death occurred following DLI-triggered GVHD complicated by pulmonary infection. These findings suggest that with contemporary supportive care, including letermovir prophylaxis and judicious use of DLI, TRM with this platform could be further reduced.

Several limitations must be acknowledged. This is a single-center, retrospective analysis of a small cohort (n = 10), which precludes definitive conclusions and limits statistical power. The long enrollment period (2012–2025) introduces heterogeneity in supportive care practices. The absence of a comparator arm prevents direct comparison with PTCy-based approaches. Additionally, the αβ T-cell depletion platform requires specialized graft processing facilities and is more resource-intensive than unmanipulated transplant approaches, which may limit its widespread applicability. Despite these limitations, the results of this study are compelling. The combination of 100% PFS, low GVHD incidence without pharmacological immunosuppression, and manageable TRM in a cohort of heavily pretreated patients with predominantly active disease at transplant suggests that αβ TCR/CD19 depletion represents a safe and effective transplant platform for R/R Hodgkin lymphoma.

In conclusion our approach warrants further investigation in prospective, multicenter studies comparing it directly with PTCy-based haploidentical transplantation and may be particularly suited for patients bridged with checkpoint inhibitors, those with high comorbidity burden who would benefit from avoidance of post-transplant immunosuppression, or those requiring urgent transplantation when a matched donor is unavailable.

Declarations

AUTHORSHIP CONTRIBUTIONS.

Conceptualization and Design, L.P. and F.A.; Collection and Processing, A.R. L.P. ; Clinical care team, L.P. A.R. B.C. F.A., Formal Analysis L.P. G.R. S.B.; Investigation, A.M. and G.R.; Writing – Review & Editing, L.P. and G.R.. All the authors read and discussed the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this topic

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Figures

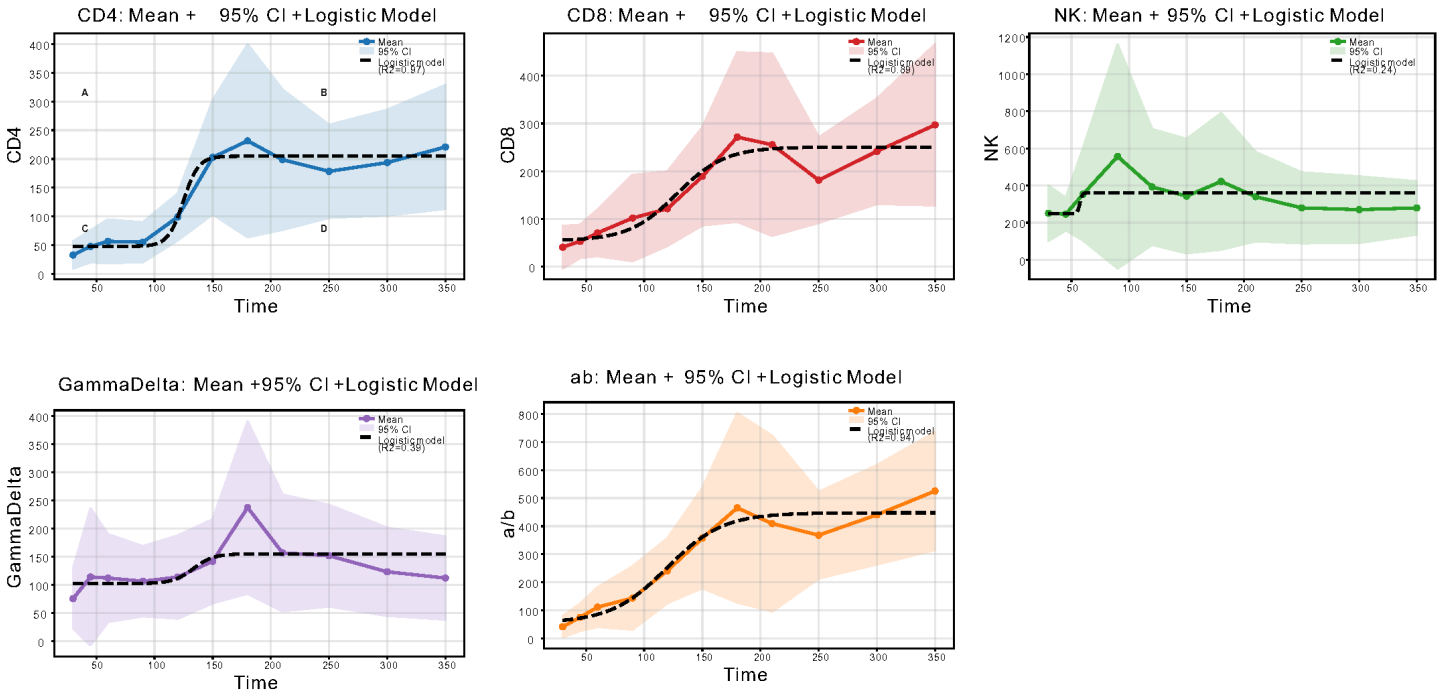
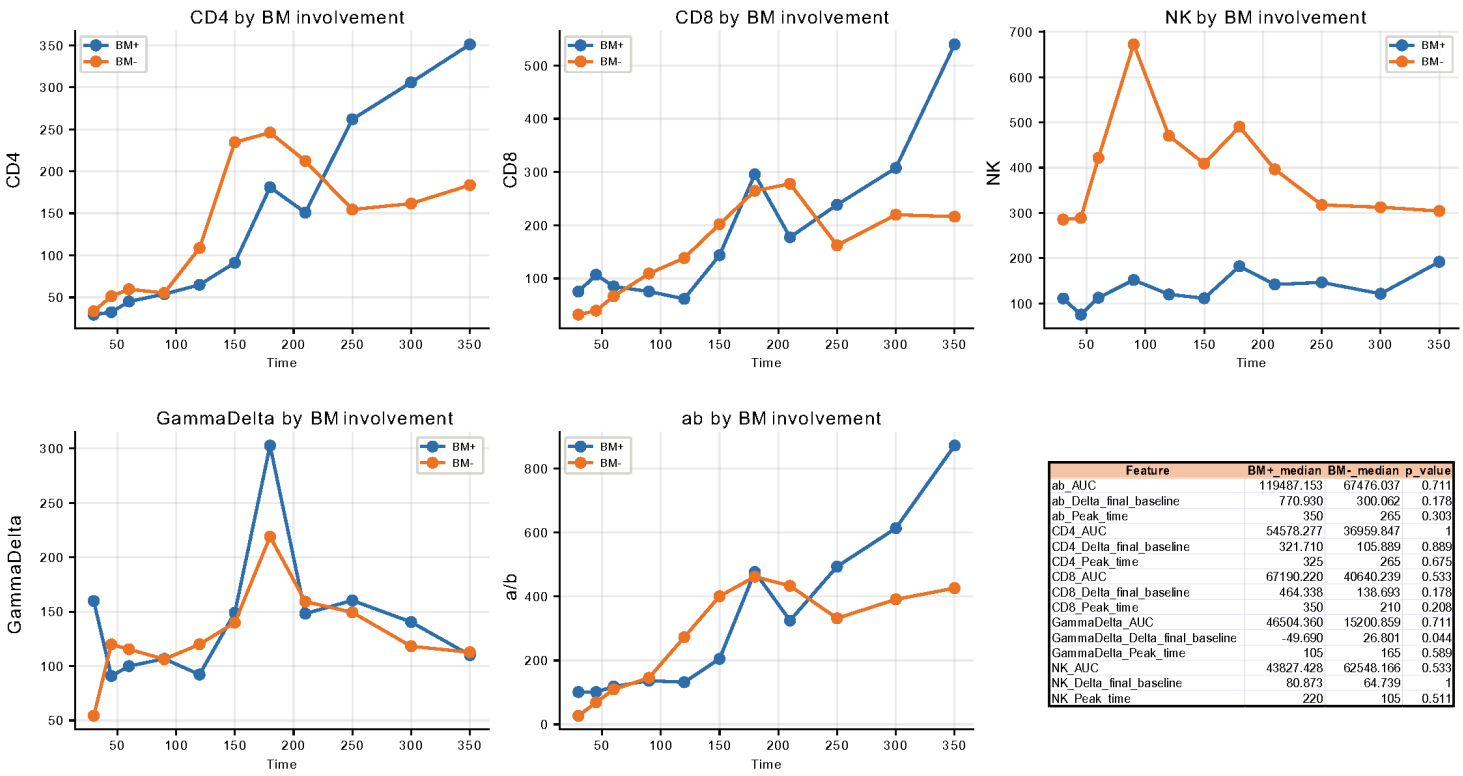


Figure 1. Longitudinal dynamics of immune cell populations.

Figure 1

Longitudinal dynamics of immune cell populations.

Mean values (solid lines) and 95% confidence intervals (shaded areas) of CD4, CD8, NK, γδ (gamma-delta), and a/b lymphocyte populations are shown over time (days 30–350). All populations exhibit heterogeneous temporal dynamics, with a general increase observed after day 120. NK and ab populations show higher absolute values and broader variability, while CD4 and CD8 display a more gradual increase. γδ cells present intermediate behavior with inter-individual variability.



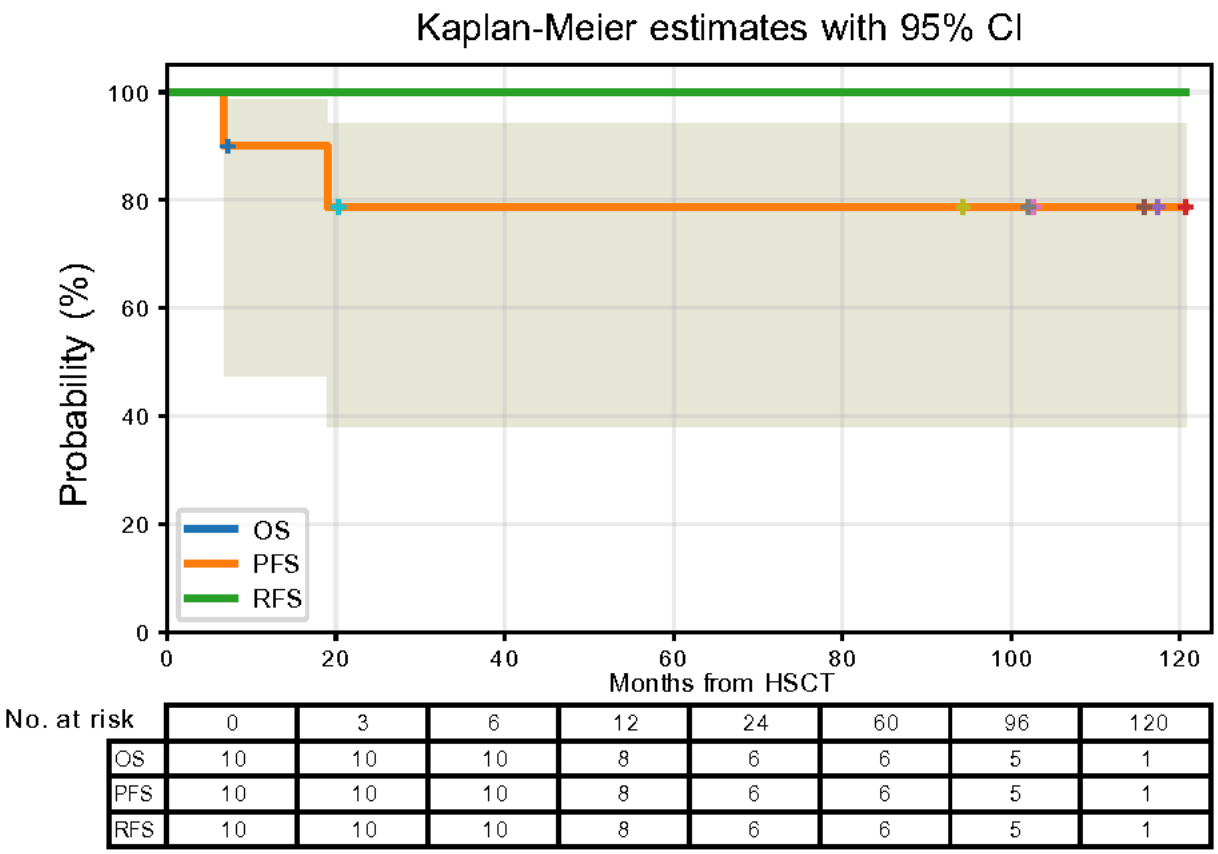
Feature	BM+_median	BM-_median	p_value
ab_AUC	119487.153	67476.037	0.711
ab_Delta_final_baseline	770.930	300.062	0.178
ab_Peak_time	350	265	0.303
CD4_AUC	54578.277	36959.847	1
CD4_Delta_final_baseline	321.710	105.889	0.889
CD4_Peak_time	325	265	0.675
CD8_AUC	67190.220	40640.239	0.533
CD8_Delta_final_baseline	464.338	138.693	0.178
CD8_Peak_time	350	210	0.208
GammaDelta_AUC	46504.360	15200.859	0.711
GammaDelta_Delta_final_baseline	-49.690	26.801	0.044
GammaDelta_Peak_time	105	165	0.589
NK_AUC	43827.428	62548.166	0.533
NK_Delta_final_baseline	80.873	64.739	1
NK_Peak_time	220	105	0.511

Figure 2. Longitudinal dynamics of immune cell populations stratified by bone marrow involvement at diagnosis

Figure 2

Longitudinal dynamics of immune cell populations stratified by bone marrow involvement at diagnosis.

Median values of CD4, CD8, NK, γδ (gamma-delta), and a/b lymphocyte populations are shown over time (days 30–350) stratified by bone marrow involvement at diagnosis. Table represents statistical analysis associated to each graph.



No. at risk	0	3	6	12	24	60	96	120
OS	10	10	10	8	6	6	5	1
PFS	10	10	10	8	6	6	5	1
RFS	10	10	10	8	6	6	5	1

Figure 1. Kaplan–Meier estimates of OS, PFS, RFS

Figure 3

Kaplan–Meier estimates of overall survival, progression-free survival, and relapse-free survival.

Kaplan–Meier curves for overall survival (OS), progression-free survival (PFS), and relapse-free survival (RFS) after HSCT are shown with 95% confidence intervals and numbers at risk. PFS was defined as relapse, progression, or death, whereas RFS was defined as relapse/progression only. No relapse or progression was observed during follow-up; therefore, RFS remained 100%, while OS and PFS were affected by two non-relapse deaths.