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Reduced intensity hematopoietic cell transplant using best available donor and post-transplant cyclophosphamide is safe and curative for transfusion-dependent thalassemia

Short Title: Reduced intensity HCT with PTCy for Thalassemia

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Abstract

Over 300,000 live births annually are affected by hemoglobinopathies worldwide. Of these, transfusion-dependent thalassemias (TDTs) result in severe phenotypes that significantly impact quality of life, shorten life expectancy, and incur high lifetime healthcare costs driven by recurrent transfusions and chelation therapy. Allogeneic hematopoietic cell transplantation (allo-HCT) is curative but limited by donor availability, regimen toxicity, graft-versus-host disease (GVHD), and transplant-related mortality. We present our single-center experience of TDT patients who underwent allo-HCT using a reduced-intensity conditioning (RIC) regimen (anti-thymocyte globulin, fludarabine, cyclophosphamide, total body irradiation) and post-transplant cyclophosphamide (PTCy)-based GVHD prophylaxis. Twenty-five consecutive children and young adults (ages 2-23 years, median 9) with TDT (beta-thalassemia major n=21, hemoglobin E-beta thalassemia n=3, hemoglobin D-beta thalassemia n=1) underwent HCT from July 2013 to November 2024. Bone marrow was the most common graft source (n=23, 92% of all patients), and haploidentical donors were the most common donor type (n=19, 76% of all patients). Neutrophil and platelet recovery occurred at median 21 days (range 13-38) and 26 days (range 16-88), respectively. Five patients (20%) developed grade I-II acute GVHD; none developed grade III-IV acute GVHD. Two (8%) developed mild chronic GVHD. At a median follow-up of 5.6 years (range 0.7-10), 5-year overall survival was 100%, transfusion-free survival 96%, and thalassemia-free-GVHD-free survival 96% - one patient experienced near-complete loss of donor chimerism with return of the TDT phenotype. Our RIC platform with best-available donor and PTCy-based GVHD prophylaxis significantly reduces post-HCT complications while providing durable transfusion independence, offering a proven, accessible, curative option for TDT.

Introduction

It is estimated that over 300,000 live births annually are affected by inherited hemoglobinopathies [1]. Transfusion-dependent thalassemias (TDTs), including beta (β)-thalassemia major, result from mutations in the β-globin gene causing imbalanced globin chain production and ineffective erythropoiesis. This leads to extramedullary hematopoiesis, bone deformities, growth impairment, and life-long transfusion dependence with associated risks of viral infections and secondary iron overload causing organ dysfunction [2, 3]. Consequently, TDTs incur high lifetime healthcare costs driven primarily by the need for frequent transfusions and chelation therapy [4].

Allogeneic hematopoietic cell transplantation (allo-HCT) remains the only widely established therapy for TDT patients of all ages, offering transfusion independence, normalization of quality of life, resolution of iron burden, and reversal of end-organ damage compared to conventional treatment with regular transfusions and chelation [3, 5, 6]. Historically, outcomes have been influenced by donor type, stem cell source, conditioning regimen intensity, and pre-existing comorbidities from chronic iron overload. While unaffected, HLA-matched, sibling donors have historically yielded the best outcomes, only ~30% of patients have such donors available [7-11]. Alternative fully matched unrelated donors are limited by registry availability and even when available, the outcomes are inferior to allo-HCT using related donors due to higher rates of transplant-related mortality (TRM), graft rejection, infection, and graft-vs-host disease (GVHD) [3, 9, 12-14]. Conditioning intensity also significantly impacts outcomes. Myeloablative conditioning (MAC) regimens carry substantial risks: the Pesaro group demonstrated that suboptimal chelation and liver fibrosis increase graft rejection and TRM [15-17]. Hence, adolescents and adults with pre-existing end-organ damage face elevated short-term TRM, while children risk long-term sequelae including neurodevelopmental impairment, infertility, and secondary malignancy [6, 13, 18-20]. Reduced intensity conditioning (RIC) lessens these adverse events but historically is associated with increased rejection risk due to expanded host hematopoiesis and allo-immunization from transfusions [12, 21, 22].

Multiple strategies to improve allo-HCT outcomes have yielded suboptimal results: Augmented GVHD prophylaxis with MAC remained problematic; abatacept improved outcomes but incidence of endothelial injury syndromes remained 16% with busulfan-based myeloablation in the abatacept group and 64% in the non-abatacept group [23]. Alemtuzumab addition showed benefit primarily in children under 12 receiving matched related grafts [24] while alemtuzumab-containing RIC with unrelated donors resulted in significant acute and chronic GVHD with six deaths among 33 patients [12]. Haploidentical approaches using alpha-beta T-cell depletion improved count recovery but were associated with 14% graft failure, extensive chronic GVHD, and delayed immune reconstitution [25]. PTCy-based haploidentical transplantation showed excellent engraftment and survival, though requiring MAC and several blocks of immunosuppression pre-HCT [10]. Finally, a recent randomized trial demonstrated that lower-dose ATG (8 vs 10 mg/kg) performed comparably for overall survival in TDT patients undergoing MAC with matched sibling donors [26].

Hence, while curative potential exists, knowledge gaps remain regarding outcome optimization [13, 20]. A safer, more accessible strategy that broadens donor availability, promotes durable engraftment, achieves low GVHD and TRM rates, and fosters robust immune reconstitution represents a critical unmet need. RIC platforms incorporating PTCy for immunologic tolerance and GVHD prophylaxis have been developed for various disorders, enabling safe utilization of haploidentical grafts with near-universal donor availability, even in frail patients previously unsuitable for HCT [20, 27-29]. Herein, we present our center's experience with allo-HCT for pediatric and young adult patients with TDTs over the past decade.

Methods

Design

This was a retrospective single-center study performed via chart review of electronic records in accordance with the Declaration of Helsinki and the International Conference on Harmonization

Guidelines for Good Clinical Practice. The study was approved by the Johns Hopkins Institutional Review Board.

Subjects

All subjects who received an allo-HCT with the indication of TDT between July 2013 and November 2024 were included. Data collection was completed in September 2025. Five of the 25 patients were previously published [20]; updated follow-up on these 5 patients along with outcomes data on 20 additional subjects are included here.

Data Collection

Data collected included: patient and donor demographics, transplantation parameters, survival, transfusion frequency, time to hematopoietic recovery and transfusion independence, donor chimerism, post-transplant toxicities, the incidence and grade of acute and chronic GVHD, and TRM. Neutrophil recovery was defined as the first of three consecutive days of achieving a sustained peripheral blood absolute neutrophil count of >500 cells/mm^3. Platelet recovery was defined as the first of three consecutive days of achieving a sustained platelet count of >20,000 cells/mm^3 without a transfusion within the preceding seven days.

Outcomes

Primary outcomes were overall survival and red blood cell transfusion independence. Secondary outcomes were time to neutrophil and platelet recovery, donor chimerism (engraftment), along with the incidence of primary and secondary graft failure, acute and chronic GVHD [30, 31], TRM, and regimen-related toxicity (RRT), including infections, veno-occlusive disease (VOD), idiopathic pneumonia syndrome (IPS), and transplant-associated microangiopathy (TA-TMA). Mixed chimerism was defined as the presence of >5% and <100% donor cells either in the whole blood or in the CD3+ compartment. Primary graft failure was defined as lack of achievement of an absolute neutrophil count ≥500/µL by day +30 with associated pancytopenia [32]. Secondary graft failure was defined as sustained decline of donor chimerism after initial engraftment leading to the return of red blood cell transfusion

requirement consistent with the phenotype of TDT. Transfusion independence was defined as the last day post-transplantation that a platelet or red blood cell (RBC) transfusion was administered.

Supportive Care

Supportive care was provided as per our institutional guidelines. Patients who were CMV IgG positive pre-HCT or whose donor was CMV IgG positive received prophylaxis with acyclovir (500 mg/m^2) until count recovery and then underwent weekly monitoring with plasma CMV PCR until 100 days post-transplant. Starting in November 2024, acyclovir was replaced by letermovir, administered from day 0 until day +100 to patients at risk for CMV reactivation. Patients who were HSV1 or HSV2 IgG positive received prophylaxis with acyclovir (250 mg/m^2) until neutrophil recovery. All patients received antibacterial prophylaxis until neutrophil recovery and prophylaxis against *Pneumocystis jirovecii* pneumonia starting on day +30 through one-year post-HCT. Antifungal prophylaxis was administered from day 0 to day +75, most commonly with an azole antifungal agent. All patients received ursodiol (6 mg/kg divided BID) through day +90. Patients received granulocyte colony stimulating factor (G-CSF) from day +5 post-HCT until neutrophil recovery.

Results

Patient, Donor, and Transplantation Characteristics

Patient, donor, and transplantation characteristics for all groups are shown in Table 1. Pesaro classification [17] and risk factors for those patients who underwent liver biopsy are listed in Table 2. Iron overload was assessed by serum ferritin along with liver iron concentration as assessed by MRI (FerriScan®) and/or liver biopsy in most of the patients. All patients received aggressive iron chelation in the months leading up to HCT in an attempt to reduce ferritin below 1000 ng/ml if possible [33]. All patients received hydroxyurea for approximately 60 days leading up to transplant, at a dose of 15-30 mg/kg. Fertility preservation was discussed with all patients and families pre-HCT. When appropriate, procedures included sperm banking, oocyte

cryopreservation or ovarian tissue cryopreservation. As of 2021, male patients underwent testicular shielding during TBI.

Unmanipulated bone marrow was the most common graft source (n=23, 92% of all patients), and haploidentical donors were the most common donor type (n=19, 76% of all patients). Two patients received G-CSF mobilized peripheral blood stem cell grafts. Most of the patients received an ABO compatible graft (n=22, 88%), three had a minor mismatch, and none had a major ABO mismatch. None of the patients had donor-specific antibodies (1 patient had significant antibodies against available related donors and thus received an unrelated donor graft).

The conditioning regimen for the majority of patients (n=23/25, 92%) was our standard institutional reduced intensity platform for non-malignant disorders using rabbit ATG (0.5 mg/kg/dose once daily IV on day -9, 2 mg/kg/dose once daily IV on day -8, -7, cumulative dose 4.5 mg/kg), fludarabine (30 mg/m^2/dose once daily IV on days -6 to -2, cumulative dose 150 mg/m^2), cyclophosphamide (14.5 mg/kg/dose once daily IV on days -6 and -5, cumulative dose pre-HCT 29 mg/kg) and total body irradiation (TBI) of 400 cGy delivered in a single dose on day -1. One patient received 200 cGy of TBI as this standard approach was being developed. One patient, who was receiving a second transplant, did not receive ATG. This was the first attempt at a curative HCT for all but two patients who had undergone previous HCT attempts at other institutions before presenting to our center. GVHD prophylaxis was delivered via PTCy with mesna (50 mg/kg once daily IV on days +3 and +4, cumulative dose post-HCT 100 mg/kg), mycophenolate mofetil (15 mg/kg/dose TID, maximum daily dose 3 gm, day +5 to +35), and sirolimus (day +5 to +365, goal trough 5 – 15 ng/mL).

Survival, Engraftment, and Chimerism

Table 3 lists the outcomes of our patient cohort. Five-year overall survival was 100% and transfusion-free survival 96% with a median follow-up of 5.6 years (range 0.7 – 10); one patient experienced sudden death of undetermined etiology following a brief viral illness seven years

post-HCT, at which point they were transfusion independent, fully engrafted with evidence of immune reconstitution, and with no known HCT-related complications. All patients achieved primary hematopoietic cell engraftment, with a median time to recovery for neutrophils of 21 days (mean 21, range 13 – 38 days) and 26 days for platelets (mean 30, range 16 – 88 days, Figure 1A). Median time to transfusion independence was 25 days for red cells (range 11 – 168) and 19 days for platelets (range 10 – 133, Figure 1B). Average hemoglobin level approximately 6 months after last administered red cell transfusion was 11.5 g/dL (median 11.4, range 8.2 – 15.2) in the whole cohort, 12.1 g/dL (median 11.9, range 8.9 – 15.1) in patients with full donor whole blood chimerism, and 10.2 g/dL (median 9.9, range 8.2 – 15.2) in patients with mixed chimerism. As shown in Figure 2A, 100% of patients achieved high mixed or full donor chimerism in the whole blood compartment by day +30 and 84% maintained high mixed or full donor chimerism at the time of last follow-up. Similar findings were noted in the T-cell (CD3+) compartment; high-level mixed or complete donor chimerism was observed in 88% at day +30 and 88% at time of last follow-up (Figure 2B). The single patient who received 200 cGy of TBI was noted to have down-trending whole blood donor chimerism with complete loss of donor T-cell chimerism following initial engraftment. In response, the patient received equine ATG post-HCT (40 mg/kg/dose once daily IV for 4 days between day +116 to +119) with the intent of eradicating returning host lymphocytes. This resulted in re-establishment of donor chimerism in the T-cell compartment with subsequent improvement of donor chimerism in both compartments; at last follow-up donor chimerism was 50% in the CD3 compartment and 77% in the whole blood 7 years out from transplantation. Secondary graft failure occurred in 1 patient (4%); this patient also had undetectable donor T-cell chimerism on day +60 and +90 and received equine ATG. Despite transient improvement in T-cell chimerism, whole blood chimerism waned over several years; the patient reverted to their prior TDT phenotype with whole blood and T-cell chimerism at 5% and 8%, respectively, at the time of last follow-up 8 years post-HCT. One additional patient has retained stable mixed chimerism between 10-20% while remaining transfusion independent and asymptomatic at the time of last follow-up, 5 years post-transplantation. Immune reconstitution was assessed at six and twelve months (Supplemental Table 1). At 6 months post-HCT, the median absolute lymphocyte count (ALC)

was 1.23 K/mm^3 (0.38 – 11.72) and 70% of patients had a CD4+ count >200 cells/mm^3; by twelve months, median ALC was 1.88 K/mm^3 (1.02 – 7.70) and 100% of patients had a CD4+ count >200 cells/mm^3. Immunoglobulin levels were also restored at the one-year mark.

Graft versus Host Disease, Regimen-Related Toxicity, and Transplant-Related Mortality

At last follow-up, there were 5 cases (20%) of acute grade II GVHD: 2 patients with isolated skin GVHD (max stage 3), and 3 patients with intestinal GVHD (max stage 2, Figure 3). All patients responded to first-line therapy with systemic and/or topical corticosteroids. There were no cases of grade III-IV GVHD. Two patients were diagnosed with mild chronic GVHD limited to the skin and oral mucosa and responded to first-line therapy. Five-year thalassemia-free-GVHD-free survival (defined as: alive, transfusion-independent, and free of extended chronic GVHD) was 96% [34]. Infectious complications are listed in Supplemental Table 2. Seven patients (28%) experienced bacteremia, 1 with sepsis, and 1 patient who developed necrotizing cellulitis secondary to *Escherichia coli* upon count recovery. The patient had traveled outside the United States in the weeks leading up to HCT but otherwise had no known pre-HCT risk factors. The patient fully recovered following broad-spectrum antibiotics, surgical debridement, and ultimately placement of a skin allograft from the HCT donor. The incidence of viral reactivation/infection was low. CMV reactivation occurred in 2 patients and was treated per institutional guidelines; there was 1 case of mild presumed CMV pneumonitis. This patient had persistent fever without significant hypoxemia. Chest CT revealed new infiltrates. Bronchoalveolar lavage was negative for CMV by shell vial culture but positive by qualitative PCR and plasma viral load was below the limits of quantification. The patient was ultimately treated with ganciclovir, with resolution of fever and infiltrates on CT scan. One patient experienced adenoviremia while on immune suppression, without clear organ involvement. EBV reactivation was not observed. Non-infectious complications occurred infrequently (Table 3). All patients had evidence for significant transfusion-associated iron overload at the time of HCT (Table 2). Pesaro scoring [17] was completed on 18 of 25 patients. Eleven patients had evidence of fibrosis on liver biopsy, 10 with mild portal fibrosis, and 1 young adult patient with a multi-year history of transfusions had evidence of bridging fibrosis. Despite these findings, no

patient receiving our RIC platform developed significant liver dysfunction nor liver enzyme elevation post-transplant. One patient developed IPS and recovered fully after treatment with steroids and etanercept [35]. No patients developed TA-TMA. One patient developed Crohn's disease 1.5 years after HCT; the disease is now quiescent, and the patient is alive and well 10 years post-transplantation. In terms of late effects, 1 (4%) patient developed hypothyroidism. Two (8%) female patients who were pre-pubertal at the time of HCT had low-for-age anti-Mullerian hormone during long-term follow-up and were able to successfully undergo ovarian stimulation and oocyte retrieval during puberty, at 3 and 5 years post-HCT. Two (8%) female patients who were pubertal or post-pubertal at the time of HCT experienced premature ovarian insufficiency.

Discussion

The scientific community has made tremendous progress over the last 5 decades in the field of allo-HCT for hemoglobinopathies and other non-malignant disorders. In 1976, Dr. David G. Nathan, Professor of Pediatrics, wrote in the Western Journal of Medicine [36]: *"Bone marrow transplantation is a logical thought, and the technical aspects of it are increasingly understood […] However, a decision to carry out a bone marrow transplantation in a very young patient with thalassemia would be extraordinarily difficult in view of the fact that conservative management should offer an 18 to 20 year life span for the patient as opposed to the 40 to 50 percent risk of immediate mortality due to the transplant procedure. Obviously, if one elects to do a marrow transplantation late in the course of life span of a patient with thalassemia, sensitization by transplantation antigens present in transfused blood will most assuredly compromise any hopes for success. Furthermore, the liver disease secondary to iron overload would notably increase the hazard of immunosuppressive therapy, particularly with cyclophosphamide. Transplantation certainly presents no easy solution at this time."* Nearly half a century later, we are in the remarkable position to have almost fully addressed these aptly delineated obstacles.

The first case of a successful matched sibling HCT in a 16-month-old child with thalassemia major was reported by Thomas et al in 1982 [7], and 2 years later Lucarelli et al reported on a cohort of 13 patients, 2 of which survived and were cured [8]. Several groups have reported their experience since then, as detailed in the Introduction section. A large registry study from the European Group for Blood and Marrow Transplantation interrogated the outcomes of 1493 patients with TDT transplanted between 2000 and 2010. The majority of these were performed using an HLA-identical sibling, and at a median observation time of 2 years, overall survival and event-free survival were found to be 91% and 83%, respectively [3]. The outcomes were significantly worse for patients without a matched donor; both overall and event-free survival dropped to 68%. A 2024 meta-analysis reviewed published data on haploidentical transplants for TDT and identified 10 studies encompassing 356 patients across a variety of geographical locations, conditioning regimens, and GVHD-prophylaxis methods, with a pooled overall survival rate of 92%, thalassemia-free survival of 85%, and graft failure rate of 8% [37]. Treatment-related mortality was 7%, overall acute GVHD 30%, and grade III-IV was 9%.

More recently, preliminary results from the Thal-FAbS trial (NCT05426252) were released, describing 12 patients who were transplanted using a reduced-toxicity treosulfan-based regimen with a long pre-transplantation immunosuppression period including treatment with fludarabine and prednisone, and ATG/abatacept/sirolimus based GVHD-prophylaxis [38]. The majority of these transplants used matched sibling donors. Two haploidentical donors were included and those patients received a single dose of PTCy in combination with the agents above. The authors report that at a median follow-up time of 12 months, all patients were alive and engrafted, with low rates of GVHD. While these early results are encouraging, one must note that the approach is still within the spectrum of MAC, involves a prolonged period of immune suppression, and the experience with non-matched sibling donors is limited.

It is now widely accepted that the use of PTCy has significantly impacted the field of allo-HCT, not merely by overcoming traditional HLA-barriers in donor selection, but also via providing highly effective prophylaxis against acute and chronic GVHD across multiple disease indications,

conditioning platforms, and graft sources [28, 39, 40]. The mechanisms through which this approach modulates the immune response remain to be fully elucidated but may include the induction of alloreactive T-cell dysfunction, indirect effects on regulatory T-cells, and the expansion of myeloid-derived suppressor cells [41]. In our thalassemia cohort, the incidence of graft rejection is notably low, despite the disease's known propensity for mixed chimerism and graft loss, especially with the use of RIC approaches. We hypothesize that the TBI dose used in our conditioning platform contributed substantially to the favorable outcomes. In 2012 Bolaños-Meade et al reported the experience of using a NMA approach with PTCy in 17 patients with sickle cell disease, with 100% survival but a graft failure rate of 43% [42]. Increase of the TBI dose from 200 to 400 cGy in the same platform again resulted in excellent survival and reduction of graft rejection incidence to 6% [20]. This same approach has been successful in reducing the graft failure risk in patients with treatment-naïve, severe aplastic anemia [40]. While risk of secondary malignancy with TBI is always a concern, the risk with low dose TBI is not substantially higher than with chemotherapy alone [43]. Of note, two of our patients experienced declining whole-blood donor chimerism with complete loss of donor T-cell chimerism, findings typically associated with impending graft rejection. In both cases, treatment with equine ATG was followed by recovery of donor chimerism in both the whole-blood and T-cell compartments; in one case the response waned over several years, while the other has been sustained and stable for over five years. We hypothesize that following loss of donor T-cell chimerism, ATG specifically targeted residual host lymphocytes thereby permitting re-expansion of marrow-derived donor T-cells required to support longer-term donor hematopoiesis. We note that in our cohort, two patients experienced ovarian insufficiency; another two who were pre-pubertal at the time of HCT retained sufficient ovarian function to undergo successful oocyte retrieval later in puberty. It is a well-known phenomenon that prepubertal status provides a relative protective effect to chemotherapy and radiotherapy effects on ovarian tissue [44] and future ovarian shielding strategies may therefore be of particular importance for pubertal and post-pubertal patients. However, this has not yet been adopted as standard practice at our institution due to concern that shielding of the abdominal cavity could result in under-dosing adjacent marrow.

At present, allo-HCT remains the only therapy with established long-term outcome data. Luspatercept, approved for adult TDT patients in 2019, reduces transfusion burden but is non-curative, mechanistically not fully understood, and not well studied in children [45]. Gene therapy offers an autologous alternative: betibeglogene autotemcel (approved 2022) adds a functional β-globin gene via lentiviral transduction but appears non-curative in most patients with severe β-null genotypes, with limited long-term data [46-48], while exagamglogene autotemcel, a CRISPR-Cas9 therapy targeting BCL11A, achieved transfusion independence in most patients but with only short follow-up to date [49, 50]. Although these approaches avoid allogeneic donors, GVHD, and post-transplant immunosuppression, they still require myeloablative conditioning, with attendant risks of infertility and secondary malignancy, and are not suitable for patients with existing end-organ damage. Additionally, high cost, limited geographic availability, and need for specialized infrastructure remain substantial barriers to widespread use [51, 52].

The data presented herein have resulted in an evolution in our approach to this patient population. In 2013, busulfan-based MAC matched-related donor HCT was standard practice. Following initiation of an institutional protocol using non-myeloablative conditioning in 2006, we augmented the TBI dose from 200 cGy to 400 cGy in 2014. Based on improved outcomes this platform has ultimately become our institutional standard [20]. Our RIC allo-HCT platform using PTCy-based GVHD prophylaxis represents a safe, feasible, and effective curative therapy for pediatric, adolescent, and young adult patients with TDT regardless of donor type. This platform appears safe and highly effective in both the upfront setting and in patients who have had prior unsuccessful HCT attempts for TDT. Importantly, this approach significantly abrogates the risk of RRT, mitigates the risk of acute and chronic GVHD, and is sufficient to provide ample donor-derived healthy erythropoiesis resulting in long-term transfusion independence. We note that no selection criteria were applied beyond standard transplantation eligibility parameters, and no referred patients were excluded. We acknowledge, however, that contemporary patients with thalassemia may be in better clinical condition than those treated historically, reflecting advances in supportive care, iron chelation, and earlier referral for transplantation.

Although gene therapy offers a new frontier, the practical challenges of apheresis, manufacturing, busulfan-based conditioning, and extreme costs persist. Importantly, as approaches to allo-HCT become safer and the incidence of GVHD (and especially severe acute and chronic GVHD) is minimized, the cost disparity between gene therapy and HCT will only widen [52]. Conclusions drawn from our results are limited by the single center, retrospective design of the study, the modest cohort size, and the inclusion of various types of donors, stem cell sources, and conditioning regimens.

In sum, allo-HCT continues to be a reliable, cost-effective, and curative option for patients with TDT with a long-standing track record of success. All affected patients should be routinely referred for consultation to a tertiary high-volume center with experience in HCT.

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Table 1. Patient, Donor, and Transplantation Characteristics

Patients	**N=25**
Sex	
Male	12 (48%)
Female	13 (52%)
Median age at transplantation, years (range)	9.3 (2.6-23.5)
Diagnosis	
Hemoglobin β^{+}/β^{0} or β^{0}	21 (84%)
Hemoglobin β-E	3 (12%)
Hemoglobin β-D	1 (4%)
CMV serology (Donor/Recipient)	
-/-	3 (12%)
+/-	5 (20%)
+/+	15 (60%)
-/+	2(8%)
ABO compatibility	
Compatible	22 (88%)
Minor mismatch	3 (12%)
Major mismatch	0 (0%)
Conditioning	
ATG/flu/cy/TBI 400 cGy	23 (92%)
ATG/flu/cy/TBI 200 cGy	1 (4%)
Flu/cy/TBI 400 cGy	1 (4%)
Donor characteristics	
Matched related donor	3 (12%)
Matched unrelated donor (10/10)	2 (8%)
Mismatched unrelated donor (7/10)	1 (4%)
Haploidentical	19 (76%)
Median donor age at time of donation, years (range)	28 (9-52)
Stem cell source	
Bone marrow	23 (92%)
Peripheral blood	2 (8%)
Infused cell dose, median (range)	
Bone marrow - Total nucleated cells/kg x10^8	7.1 (3.7-15.6)
Peripheral blood - CD34+ cells/kg x10^6	9.2 (8.4-10)

ATG = antithymocyte globulin, flu = fludarabine, cy = cyclophosphamide, TBI = total body irradiation

Table 2. Pre-Transplant Iron Overload Evaluation & Pesaro class

Modality	Median (range)
FerriScan [1] (reference range 0.17-1.8 mg/g)	
Average liver iron concentration	3.7 (1.3-23.8)
Biopsy [2]	
Liver iron (reference range 200-1600 ug/g dry weight)	1719 (54-7801)
Iron index (reference range <1 umol/g/year)	3.7 (0-42.5)
Baseline ferritin (reference range 13-150 ng/mL)	1100 (343-3651)
Pesaro Class [3]	**N**
1	9
2	9
3	0
Pesaro Risk Factors [3]	**N**
Hepatomegaly	0
Fibrosis	11
Mild portal fibrosis	10
Bridging fibrosis	1
Irregular Chelation	0

[1]Data available on 23/25 patients.
[2]Data available on 17/25 patients.
[3]Data available on 18/25 patients.

Table 3. Transplant-related Outcomes & Complications

Outcome	N (%)
Median follow-up, years (range)	5.6 (0.7-10)
Overall survival, 5-year [1]	25 (100%)
Transfusion-free survival [1]	24 (96%)
Median time to engraftment, days (range)	
Neutrophils	21 (13-38)
Platelets	26 (16-88)
Graft failure	
Primary	0 (0%)
Secondary [2]	1 (4%)
Acute GVHD	
Grade I-II	5 (20%)
Grade III-IV	0 (0%)
Chronic GVHD	
Mild	2 (8%)
Moderate	0 (0%)
Severe	0 (0%)
Non-infectious complications	
Sinusoidal obstruction syndrome	0 (0%)
Transplant-associated thrombotic microangiopathy	0 (0%)
Idiopathic pneumonia syndrome	1 (4%)
Non-viral hemorrhagic cystitis	1 (4%)

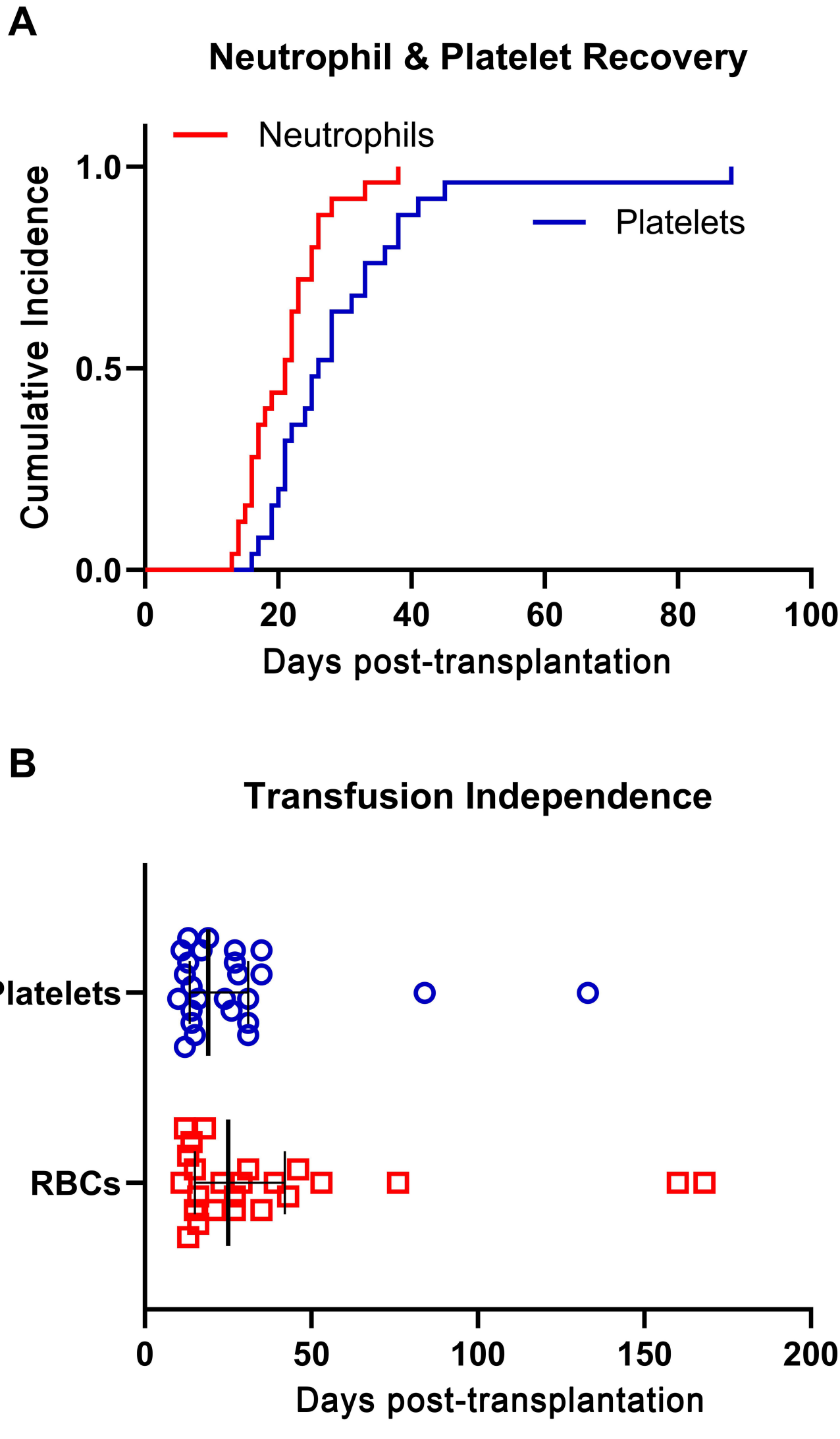
[1]*One patient passed away 7 years after transplant from an unrelated cause (sudden death of undetermined etiology after a brief viral illness), at which point they were transfusion independent and free of any major HCT-related complications.*

[2]*One additional patient (who had received 200 cGy of TBI as part of their conditioning) developed low level mixed chimerism; administration of equine ATG at day +116 post-transplant reversed the decreasing donor chimerism trend and resulted in long-term stable mixed chimerism with transfusion independence.*

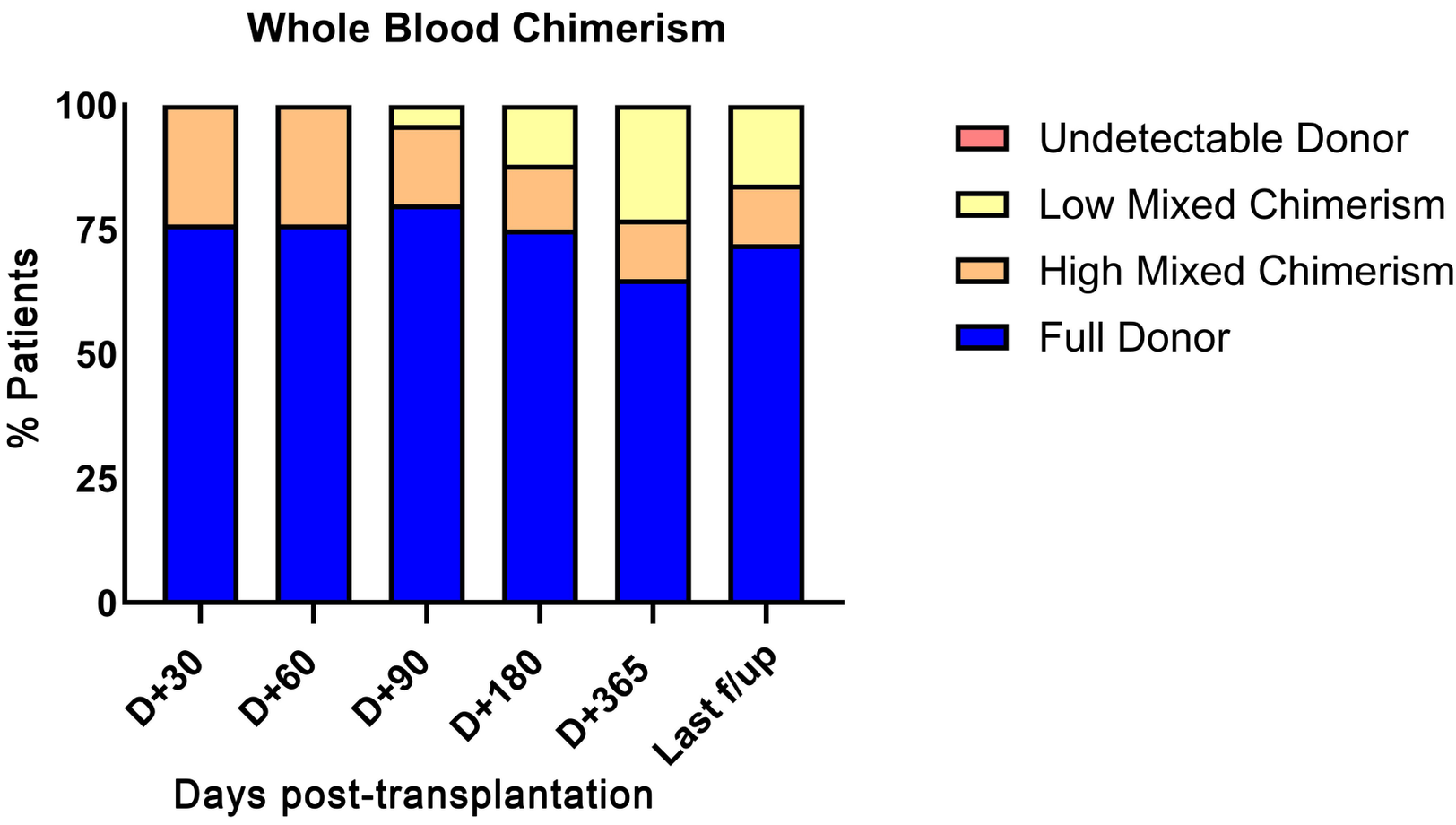
Figure 1. Cumulative incidence of neutrophil and platelet recovery and time to transfusion independence. Transfusion to independence shown as the last day post-transplantation that a platelet or red blood cell (RBC) transfusion was administered. One patient did not require RBCs peri-transplant. Another required additional transfusions starting two years after transplant due to progressive secondary graft failure (not depicted). Median time to transfusion independence was 25 days for red cells (range 11-168) and 19 days for platelets (range 10-133).

Figure 2. Chimerism in peripheral blood and CD3-sorted cells. Available donor chimerism data at each time point in peripheral whole blood (A) and CD3-sorted cells (B) categorized as follows: full donor chimerism (≥95%), high mixed chimerism (≥50% - 94%), low mixed chimerism (<50%), undetectable (0%). In (B), the two patients with undetectable donor chimerism at day +60 and/or day +90 received equine ATG; one had reversal to stable mixed chimerism, while the other progressed to secondary graft failure (with low level donor chimerism at last follow-up but return of the thalassemia phenotype).

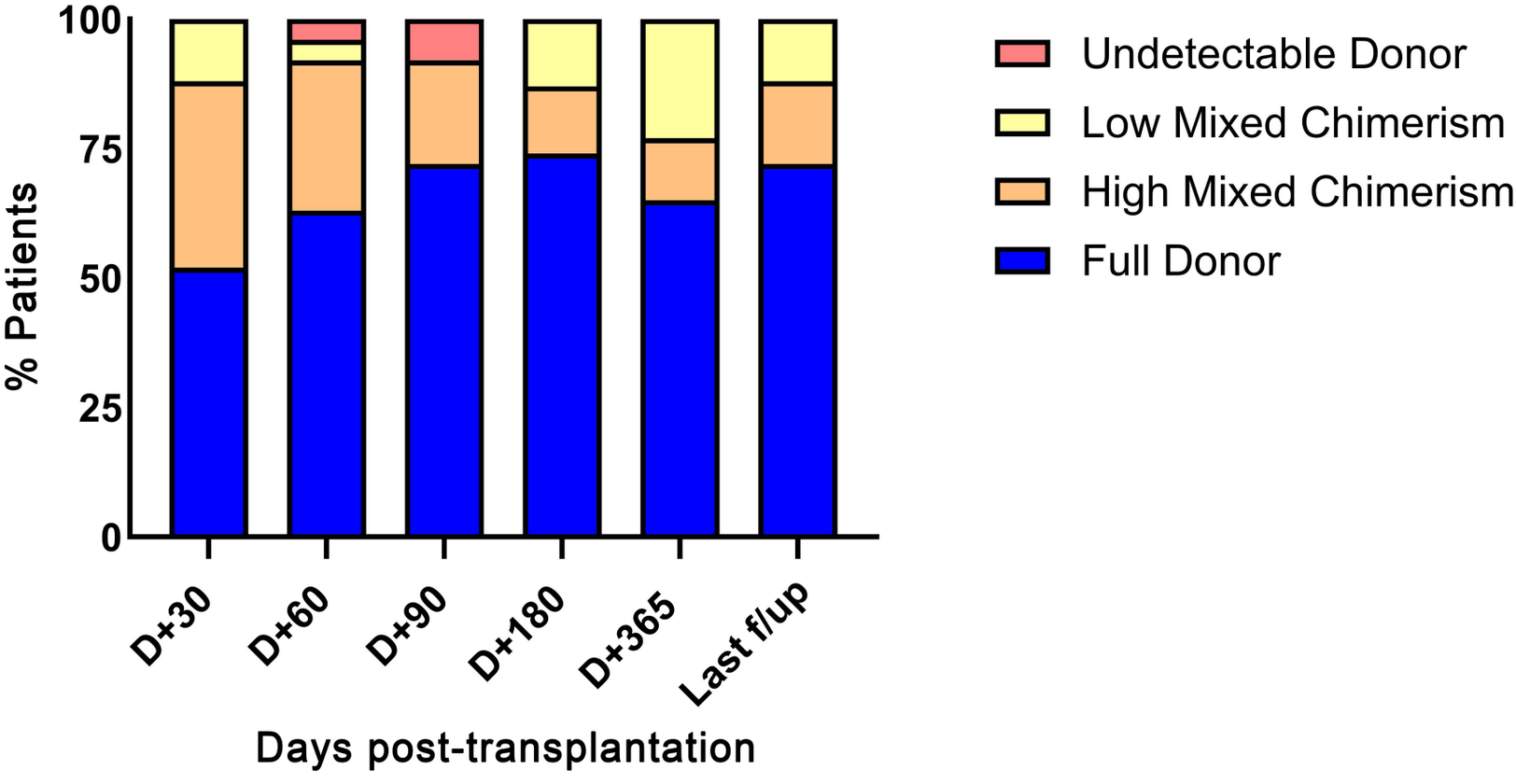
Figure 3. Cumulative incidence of acute and chronic GVHD. Cumulative incidence of acute (A) and chronic (B) GVHD; all cases of acute GVHD were grade I-II, and chronic GVHD was limited to mild.

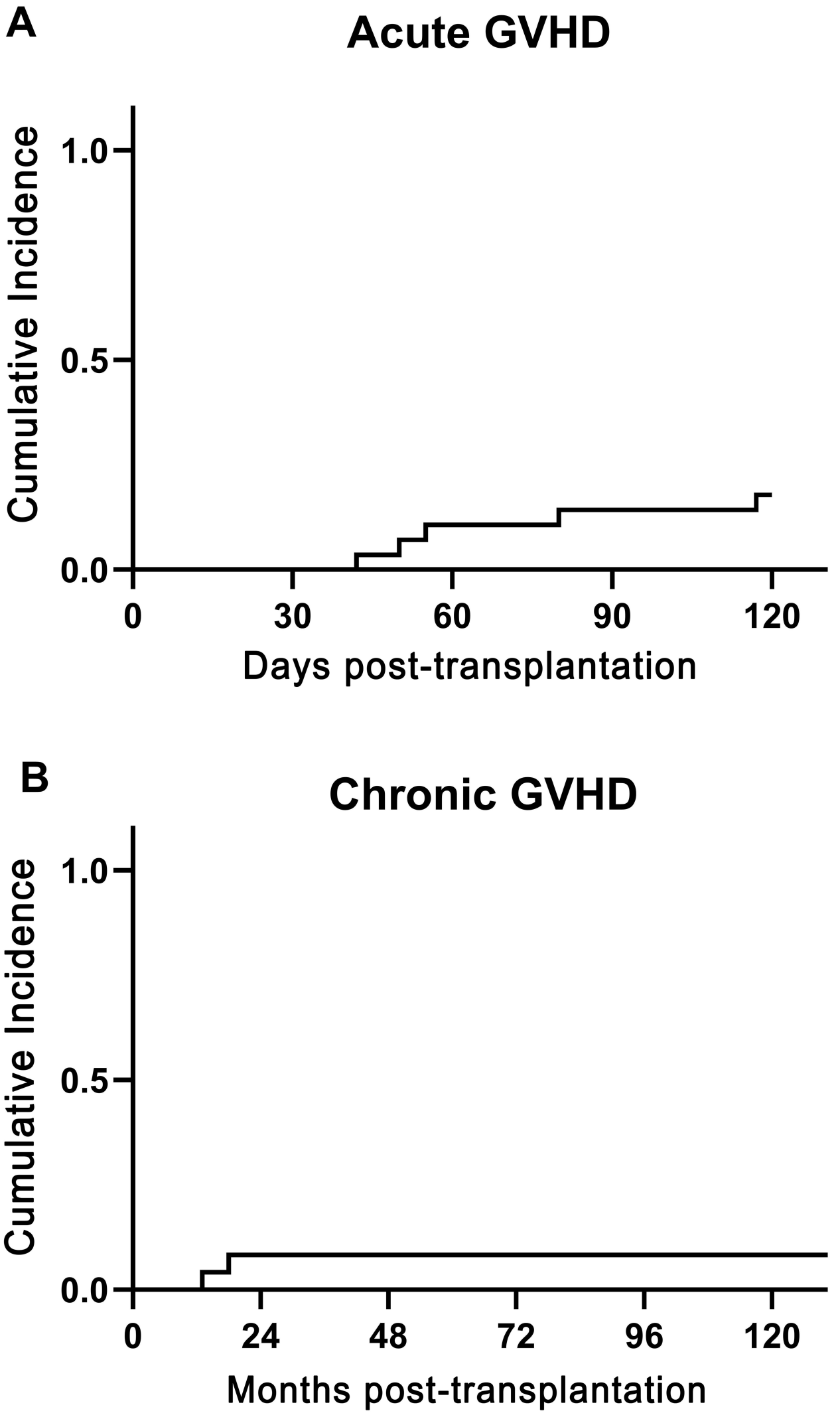

A
Neutrophil & Platelet Recovery
Neutrophils
Platelets
Cumulative Incidence
1.0
0.5
0.0
0
20
40
60
80
100
Days post-transplantation
B
Transfusion Independence
Platelets
RBCs
0
50
100
150
200
Days post-transplantation

A


Whole Blood Chimerism
100
75
50
25
0
% Patients
D+30
D+60
D+90
D+180
D+365
Last f/up
Days post-transplantation
Undetectable Donor
Low Mixed Chimerism
High Mixed Chimerism
Full Donor

B


T-cell Chimerism
100
75
50
25
0
% Patients
D+30
D+60
D+90
D+180
D+365
Last f/up
Days post-transplantation
Undetectable Donor
Low Mixed Chimerism
High Mixed Chimerism
Full Donor


A
Acute GVHD
Cumulative Incidence
1.0
0.5
0.0
0
30
60
90
120
Days post-transplantation
B
Chronic GVHD
Cumulative Incidence
1.0
0.5
0.0
0
24
48
72
96
120
Months post-transplantation

Supplemental Table 1. Immune Reconstitution Data

Lymphocyte Counts (reference range)	**Median (range)**
Patient ALC pre-HCT (1.30-10.10 K/mm^3)	2.98 (1.40-6.06)
Donor ALC	2.15 (1.26-5.18)
6 months post-HCT [1]	
ALC	1.23 (0.38-11.72)
CD3+	0.80 (0.26-9.49)
CD4+	0.27 (0.15-3.68)
CD8+	0.51 (0.06-5.72)
CD19+	0.29 (0.01-1.32)
CD16+56+	0.17 (0.04-0.82)
12 months post-HCT [1]	
ALC	1.88 (1.02-7.70)
CD3+	1.27 (0.65-5.44)
CD4+	0.64 (0.26-2.30)
CD8+	0.65 (0.19-3.28)
CD19+	0.43 (0.14-2.08)
CD16+56+	0.21 (0.07-0.54)
Immunoglobulin Levels (reference range)	**Median (range)**
6 months post-HCT	
IgG (575-1,375 mg/dL)	690 (409-1796)
IgA (50-250 mg/dL) [2]	98 (13-196)
IgM (35-150 mg/dL) [2]	47 (11-119)
12 months post-HCT	
IgG [3]	871 (434-1696)
IgA [4]	66 (26-416)
IgM [4]	38 (26-165)

ALC = absolute lymphocyte count, HCT = hematopoietic cell transplant

[1]Data available on 20/25 patients.
[2]Data available on 11/25 patients.
[3]Data available on 19/25 patients.
[4]Data available on 11/25 patients.

Supplemental Table 2. Infectious Complications

Infectious Complication	N (%)
Bacterial	
Sepsis	1 (4%)
Bacteremia	7 (28%)
Other serious bacterial infection: cellulitis	1 (4%)
Other [1]	10 (37%)
Viral	
Cytomegaloviremia	1 (4%)
CMV disease (pneumonitis)	1 (4%)
Adenoviremia	1 (4%)
EBV	0 (0%)
BK cystitis (non-hemorrhagic)	4 (16%)
HSV gingivostomatitis	2 (8%)
Other [2]	9 (36%)
Fungal	
Invasive fungal infection	0 (0%)
Other: thrush, Candida esophagitis, Candida otitis	3 (12%)

[1]*Includes sinusitis, otitis media, dental infection, lower urinary tract infection, Helicobacter pylori gastritis, Clostridium difficile enterocolitis.*

[2]*Includes rhinovirus, parainfluenza, human metapneumovirus, coronavirus, RSV, VZV reactivation.*