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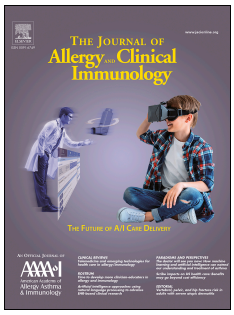

International retrospective study of allogeneic hematopoietic cell transplantation for activated PI3K-delta syndrome

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ABSTRACT

Background:

Activated phosphoinositide 3-kinase (PI3K) delta syndrome (APDS) is a combined immunodeficiency with a heterogeneous phenotype considered reversible by allogeneic hematopoietic cell transplantation (HCT).

Objective:

We sought to characterize HCT outcomes in APDS.

Methods:

Retrospective data was collected on 57 APDS1/2 patients (median age 13 years, range 2-66) who underwent HCT.

Results:

Pre-HCT comorbidities such as lung, gastrointestinal, and liver pathology were common, with hematologic malignancy in 26%. With 2.3 years median follow-up, 2-year overall and graft failure-free survival probabilities were 86% and 68%, respectively, and did not differ significantly by APDS1 vs 2, donor type, or conditioning intensity. The 2-year cumulative incidence of graft failure following first HCT was 17% overall but 42% if mTOR inhibitors (mTORi) were used in the first year post-HCT, compared to 9% without mTORi. Similarly, 2-year cumulative incidence of unplanned donor cell infusion was overall 28%, but 65% in the context of mTORi receipt and 23% without. Phenotype reversal occurred in 96% of evaluable patients, of whom 17% had mixed chimerism. Vulnerability to renal complications continued post-HCT, adding new insights into potential non-immunologic roles of PI3K not correctable through HCT.

Conclusions:

Graft failure, graft instability and poor graft function requiring unplanned donor cell infusion were major barriers to successful HCT. Post-HCT mTORi use may confer an advantage to residual host cells, promoting graft instability. Longer term post-HCT follow-up of more patients is needed to elucidate the kinetics of immune reconstitution and donor chimerism, establish approaches that reduce graft instability, and assess the completeness of phenotype reversal over time.

CLINICAL IMPLICATIONS

HCT for APDS patients reverses phenotype but is associated with high incidence of graft instability, regardless of conditioning intensity or donor type, which is increased by post-HCT mTOR inhibitor use.

CAPSULE SUMMARY

In 57 children and adults with APDS, 2-year overall survival post-hematopoietic cell transplantation (HCT) was 86% and did not differ by conditioning or donor. Graft instability was common, particularly if mTOR inhibitors were used post-HCT.

KEYWORDS

Primary immunodeficiency, activated phosphoinositide 3-kinase delta syndrome, lymphoproliferation, allogeneic hematopoietic cell transplantation, graft failure, mTOR inhibitor, serotherapy

ABBREVIATIONS

- APDS: activated phosphoinositide 3-kinase delta syndrome
- CMV: cytomegalovirus
- DCI: unplanned donor cell infusion
- EBV: Epstein-Barr virus
- GF: graft failure
- GFFS: graft failure-free survival
- GVHD: graft-versus-host disease
- HCT: hematopoietic cell transplantation
- HCT-CI: HCT Comorbidity Index
- HLA: human leukocyte antigen
- MAC: myeloablative conditioning
- MSD: matched sibling donor
- mTOR: mammalian target of rapamycin
- mTORi: mTOR inhibitor
- NMA: nonmyeloablative
- OS: overall survival
- PI3K: phosphoinositide 3-kinase
- RIC: reduced intensity conditioning
- RT-MAC: reduced-toxicity myeloablative conditioning
- VZV: varicella-zoster virus

INTRODUCTION

Activated phosphoinositide 3-kinase (PI3K) delta syndrome (APDS) was first described in 2013, with heterozygous mutations in PI3Kδ catalytic p110δ (*PIK3CD*) or regulatory p85α (*PIK3RA*) subunits leading to APDS1 and APDS2, respectively.[1, 2] PI3Ks are expressed in various hematopoietic cells and have important roles in T and B lymphocyte homeostasis. The heterogeneous immunological phenotype of APDS may include reduced naïve T cells, increased senescent $CD8^+$ T cells, increased transitional B cells, decreased class-switched memory B cells, and dysgammaglobulinemia.[1-6] Clinical manifestations vary, including, among others, recurrent sinopulmonary infections, enteropathy, autoimmunity, non-neoplastic lymphoproliferation, lymphoma, and impaired Epstein-Barr virus (EBV), cytomegalovirus (CMV) and varicella-zoster virus (VZV) control.[4, 5, 7]

Supportive care may include antimicrobials and/or immunoglobulin (Ig) replacement. Corticosteroids, rituximab and splenectomy may attenuate autoimmune and lymphoproliferative disease manifestations.[3, 8] The mammalian target of rapamycin (mTOR) activated downstream of PI3K has a significant role in the regulation of immune responses and therefore mTOR inhibitors (mTORi; rapamycin/sirolimus or everolimus) can ameliorate the severity of non-neoplastic lymphoproliferative disease and restore natural killer cell function.[9, 10] Selective PI3K-delta inhibitors are of interest as a more targeted treatment option and are under continued clinical investigation,[11] but the ability of these therapies to prevent the development of life- and organ-threatening complications for the lifespan of affected individuals remains to be seen. Allogeneic hematopoietic cell transplantation (HCT) offers a potential immunologic cure for APDS1/2 patients;[12, 13] however, questions remain regarding the optimal timing, intensity, and approach to HCT for APDS1/2, as well as the donor chimerism needed to durably achieve engraftment of donor cells and to prevent immunopathological manifestations of disease.[14] Herein, we present the largest international retrospective study of HCT outcomes of patients with APDS1/2 to date.

METHODS

Data Collection

We conducted an international case series study of the clinical outcomes of patients with APDS1/2 undergoing HCT. Each participating site obtained approval to contribute de-identified data as per institutional requirements, in accordance with the Declaration of Helsinki, prior to contributing data. Data transfer agreements were put in place when deemed required. The APDS European Society for Immunodeficiencies registry and European Society for Blood and Marrow Transplantation Inborn Errors Working Party were queried to identify European contributors.

Criteria for inclusion were 1) pathogenic germline *PIK3CD* or *PIK3RA* mutation and 2) receipt of HCT for APDS1/2 disease manifestations. A de-identified data query form was completed by participating physicians for each patient.

Data captured included patient demographics, pre-HCT disease manifestations and comorbidities, HCT-Comorbidity Index (HCT-CI) score,[15] prior therapies, HCT approach including conditioning drugs and intensity, graft source, dose, manipulation, and donor demographics, graft-versus-host disease (GVHD) prophylaxis approach, HCT complications, engraftment and chimerism kinetics, immune reconstitution, degree of post-HCT phenotype reversal, follow-up duration, and survival outcomes.

Endpoint definitions and captured complications are detailed in **Supplemental Table 1**.

Data were locked for analysis on July 13, 2020.

Statistical Analyses

Descriptive statistics were used for patient and HCT characteristics. Conditioning intensity was categorized as myeloablative (MAC), reduced-toxicity (RT-MAC, regimens using treosulfan as an alkylator), reduced-intensity (RIC), and non-myeloablative (NMA) based on published consensus definitions and the treating center's intent.[16, 17]

Survival curves were constructed for overall survival (OS) and graft failure (GF)-free survival (GFFS) using the Kaplan-Meier method and compared using the log-rank test.

Cumulative incidence curves were constructed using the method of Fine and Gray and compared using K-sample tests.[18] The cumulative incidences of transplant-related mortality (TRM), GF and subsequent unplanned donor cell infusion (DCI) were determined (competing risk: death), as well as $CD4^+$ T cell recovery >200/μL (competing risks: death, GF). Cumulative incidence curves were constructed for sub-groups based on conditioning intensity, use of serotherapy, human leukocyte antigen (HLA)-match, and use of mTORi post-HCT.

Survival curves, Kruskal-Wallis, and Fisher's exact tests were generated using GraphPad Prism, version 8.4.3 (Graph-Pad Software, La Jolla, CA; www.graphpad.com). Cumulative incidence curves were generated using R program, version 3.6.1 (R Core Development Team, Vienna, Austria; www.r-project.org). Results were considered statistically significant if 2-tailed $P < 0.05$.

RESULTS

Fifty-seven pediatric and adult APDS patients who received HCT (43 with *PIK3CD*, 14 with *PIK3RA* mutations) were included, all with confirmed pathogenic mutations and 20 with previously detailed HCT courses.[1, 2, 12, 13, 19-29] Patient and disease characteristics are shown in **Table 1**. All patients had clinical indications for HCT, and none were transplanted pre-emptively based on genetic diagnosis alone (**Supplemental Table 2**). For 45 (79%) patients, diagnosis had been genetically confirmed by the time of first HCT. Many had significant organ pathology entering first HCT, with median HCT-CI score of 2. History of immune cytopenias was reported in 33% of patients and hematologic malignancy in 26%. Most patients had received immunomodulating therapies before proceeding to HCT, including mTORi in 49%, rituximab in 26%, and PI3K inhibitor in 7%. Immunoglobulin therapy for immunomodulation or replacement occurred in 86% of patients prior to HCT. Active disease at time of HCT was common, including infection (51%), hepatosplenomegaly (51%), immune cytopenias (18%), or hematological malignancy (11%).

Donor, graft, and HCT platform characteristics for 66 HCTs performed are detailed in **Table 2**. Unrelated donors were the most frequent donor source (62%), with unmanipulated bone marrow as the most frequent graft source (48%). Matched sibling donors (MSD) were used in 11% of HCTs; these recipients also had a lower median HCT-CI score, 1, and younger median age, 9 years, as compared to recipients of grafts from other donor types, although these differences did not reach statistical significance. Most HCTs were either RIC (n=35, 53%) or MAC (n=24, 36%). The median age of patients receiving RIC vs. MAC/RT-MAC at time of first HCT was the same, 13 years, but age range was wider for RIC recipients, who also had higher median HCT-CI score (RIC 3, MAC 2, RT-MAC 0, $P = .05$). Most HCTs were performed using serotherapy (n=55, 83%). Adults were more likely than children to have hematologic malignancy as a HCT indication (67% vs 16%, $P = .001$), but age at first HCT did not correlate with survival.

The median follow-up was 27 months overall by the reverse Kaplan-Meier method and 26.3 months (range 1.5-220.6) from first HCT in surviving patients. The 2-year probabilities of OS and GFFS from first HCT were 86% and 68%, respectively (**Figure 1**). Differences in OS or GFFS were not statistically significant by underlying diagnosis (APDS1 vs APDS2), conditioning intensity, serotherapy choice, or donor source (**Figure 2**). Sub-cohorts with 2-year OS and GFFS probabilities of 100% included RT-MAC (n=4) or MSD (n=7) recipients, albeit limited by small numbers; in addition, one very late GF (day +1862) occurred in one RT-MAC HCT. TRM occurred due to late sepsis beyond 6 months post-HCT (n=3), early post-HCT pre-engraftment infections (n=2), viral infection (n=1), sinusoidal obstructive syndrome (SOS, n=1), and multi-organ failure in the setting of poor graft function (n=1); 63% of deaths occurred in the first 100 days post-HCT. Outcomes by number of patients transplanted at individual centers are shown in **Supplemental Table 3**.

Neutrophil engraftment occurred at median 16 days (range 11-127) and platelet engraftment at median 21 days (range 7-162). GF or graft instability and requirement for DCI occurred frequently, with 8 (14%) patients requiring a second HCT and 1 patient requiring a third HCT (**Table 3**). A total of 43 subsequent DCIs were administered to 18 (32%) patients, including 24 donor lymphocyte infusions and 10 stem cell boosts. Mixed chimerism was the most common indication for intervention (n=20), followed by poor graft function (n=10).

By competing risk analysis, the estimated probability of GF following first HCT was 10% (95% CI 4-44%) at 1 year and 17% (95% CI 8-30%) at 2 and 3 years (**Figure 3**). However, when mTORi was used within the first year after HCT (in 13 patients), it rose from 15% (95% CI 2-40%) at 1 year to 42% (95% CI 9-73%) at 2 and 3 years, compared to a stable and lower incidence of 9% (95% CI 3-20%) at 1, 2, and 3 years without mTORi use, $P = .06$, approaching statistical significance. More strikingly, the estimated probability of DCI, overall 17% (95% CI 8-28%) at 1 year and 28% (95% CI 15-42%) at 2 and 3 years, was 55% (95% CI 23-79%) at 1 year and 65% (95% CI 27-86%) at 2 and 3 years when mTORi was used, compared to 12% (95% CI 4-24%) at 1 year and 23% (95% CI 10-39%) at 2 and 3 years without mTORi use, $P = .002$. The cumulative incidence of DCI, but not GF, also differed by use of serotherapy, where serotherapy-free regimens had 1 and 3 year estimated probability of DCI of 48% (95% CI 12-78%) and 61% (95% CI 17-87%), compared to 18% (95% CI 8-30%) and 28% (95% CI 15-44%) with serotherapy-containing regimens, $P = .039$. The cumulative incidence of GF or DCI did not differ by conditioning intensity or HLA-match/relatedness.

Total T cells and $CD4^+$ T cells were slower to recover than $CD8^+$ T cells, B cells, and NK cells, with median total T cells within normal range at 1 year post-HCT and median CD4+ T cells within normal range at 1.5 years, whereas median counts for other lymphocyte subsets largely recovered by 6 months (**Figure 4**). Persistent profound T lymphopenia (total T cells range 46-155/uL) was reported in 5 patients (17% of those with available data) at 10-12 months post-HCT; 2 were receiving systemic therapy for GVHD. At 1 year, the estimated probability of $CD4^+$ T cell count recovery above 200/μL was 41% (95% CI 27-54%) and did not differ significantly by conditioning intensity or donor type. $CD4^+$ T cell recovery during the first year post-HCT was similar for serotherapy-containing and serotherapy-free regimens, although serotherapy-free HCT numbers were small and thus limited post-HCT lymphocyte subset data analyses for this group (**Supplemental Figure 1**).

Acute GVHD was reported in 22 (39%) patients, 5 post-DCI; maximum grade 3 acute GVHD occurred in 4 (7%) patients, 3 post-DCI. Chronic GVHD occurred in 9 (16%) patients. The estimated probability of grades 3-4 acute GVHD at 1 year was 29% (95% CI 3-64%) with MSD,

compared to 14% (95% CI 2-38%) for HLA-haploidentical, 0% for MUD, and 0% for UCB, P = .018. The cumulative incidence of grades 2-4 acute GVHD did not differ significantly by donor type, conditioning intensity or serotherapy use, nor did the cumulative incidence of grade 3-4 acute GVHD differ by conditioning intensity or serotherapy use. (**Supplemental Figure 2**)

Regimen-related and infectious post-HCT complications are summarized in **Table 4a**. SOS developed in 3 patients, all recipients of RIC regimens, including one patient with a history of pre-HCT EBV-related SOS who developed SOS anew, 1 patient had known prior liver pathology and likely multifactorial liver injury in the setting of sepsis, but meeting Baltimore criteria,[30] and 1 with no known baseline liver pathology. Interestingly, renal failure requiring dialysis was observed in 6 patients (11%), of whom 3 had known immune-mediated renal pathology prior to HCT. Additionally, one patient developed papillary renal cell carcinoma 1 year post-HCT, in complete remission post-cryoablation at last follow-up. Infectious complications were notable for EBV in blood requiring therapy in 11% of patients, CMV and adenovirus organ involvement in 7% and 2%, respectively, and lower respiratory tract viral infection other than CMV or adenovirus in 14%. CMV disease (one fatal) and EBV-post-transplantation lymphoproliferative disease occurred exclusively in patients who received proximal serotherapy (within 8 days of HCT), with the exception of one case of primary CMV infection (seronegative donor and recipient) after serotherapy-free conditioning. One patient developed renal failure requiring ongoing hemodialysis due to biopsy-proven, BK polyomavirus-associated nephropathy; BK nephropathy may have predated HCT, as this patient had renal insufficiency at baseline in the presence of BK viremia, but no pre-HCT renal biopsy was performed.

Of evaluable, engrafted survivors (n=47), 45 (96%) are alive and well with phenotype reversal, 8 (17%) in the setting of mixed chimerism in either whole blood or myeloid and/or $CD3^+$ compartments (**Table 4b**). One patient has continued disease manifestations (immune thrombocytopenia, hypogammaglobulinemia) over 2 years post-HCT in the setting of mixed donor chimerism (85% myeloid, 58% $CD3^+$) despite having received nine DCIs, albeit with improvement of the disseminated *Mycoplasma orale* infection which prompted HCT. Another has resolution of recurrent respiratory infections, enteropathy, CMV and EBV infection, and Ig replacement requirement but had alphaherpesvirus infections 2 years post-HCT despite prophylaxis, in the context of continued mixed chimerism (26% myeloid, 34% $CD3^+$). Four other patients have significant ongoing complications related to GVHD or chronic kidney disease in the setting of 100% donor chimerism and resolution of underlying reversible disease manifestations. Of 46 engrafted survivors with available data, 83% are off Ig replacement as of last follow-up. Of eight patients remaining on Ig replacement, six have less than 2 years follow-up so may still have evolving humoral reconstitution.

No clear correlation was noted between OS and donor chimerism. Infectious deaths occurred in the setting of full donor chimerism (n=2, at days +75 and +340), pre-engraftment (n=2, at days +6 and +17), split donor chimerism and profound lymphopenia (n=1 at day +238; 0.06% $CD3^+$, 99.6% myeloid chimerism at day +141), and mixed donor chimerism (n=1 at day +663 and attributed to prior splenectomy; prior to death had phenotype reversal despite 50% whole blood chimerism at day +515). Whole blood, myeloid, and $CD3^+$ donor chimerism trends expectedly showed a trend of more frequent mixed chimerism in patients who received RIC, but not all patients with mixed chimerism across compartments required intervention (**Supplemental Figure 3**). Of note, the ability to analyze chimerism trends and differences between platforms was limited by inter-center variability in the type of chimerism study and

frequency of assessment, as well as by lymphopenia hindering the ability to assess CD3 chimerism early post-HCT.

DISCUSSION

Since the original description of APDS in 2013,[1, 2] increased understanding of this disease has prompted interest in earlier access to definitive treatment such as HCT, necessitating improved awareness of factors that might optimize the approach to HCT. Prior reports have confirmed that HCT is potentially curative for patients with APDS1/2, but have also highlighted barriers to achieving better success rates in HCT for this disease, including high risk of graft instability, significant comorbidities, and poorly controlled infections, autoimmunity, and lymphoproliferation pre-HCT.[12, 13] Herein, we further characterized the international experience with the largest cohort of transplanted APDS1/2 patients to date and examined the relative contribution of HCT-related factors such as donor type, conditioning regimen, and post-HCT therapies to HCT outcomes in these patients.

While MSD are typically the preferred donor type in practice, no statistically significant differences in outcomes were noted based on donor type. Importantly, particularly in an autosomal dominant disease, unaffected MSD options may be particularly scarce.[31] Our findings suggest that having only MUD or HLA-haploidentical donor options should not dissuade or delay HCT for patients with APDS1/2 in need. Similarly equivalent outcomes between MUD and HLA-haploidentical HCTs for nonmalignant diseases and/or lymphoma have been reported in both TCRαβ+/CD19+ depletion-based and in posttransplantation cyclophosphamide-based platforms.[32-41]

Conditioning intensity, with historical preference for myeloablation in hard-to-engraft diseases, does not appear to be a key factor in the outcomes reported here. The high risk of graft failure or need for DCI was a consistent finding, with no statistically significant differences in the probabilities of OS, GFFS, GF, or need for DCI based on conditioning intensity. The 100% 2-year GFFS in the RT-MAC subgroup may suggest particular promise with this approach, but the outcome estimates for this subgroup are very limited by small numbers and more patient outcomes data are required to draw conclusions. Given the activated, dysregulated immune function that predominates this disease, it is not surprising that the use of serotherapy and thus host *lympho*depletion, rather than *myelo*ablation, intensity contributed to the risk of graft instability and need for DCIs in this study. The importance of robust host lymphodepletion was also demonstrated through the relationship between post-HCT mTORi use and the risk of graft instability or need for DCIs. It is known that mTORi ameliorate the function and survival of APDS patient lymphocytes by reducing T cell senescence, increasing naïve T cell percentage, and normalizing interleukin-2-mediated lymphoproliferation.[2] Thus, mTORi use early post-HCT may actually provide an undesirable survival advantage to residual host lymphocytes, thus mediating graft instability. Whether these results are applicable to other clinical HCT situations, such as somatic PI3K mutations in lymphoma patients without underlying primary immunodeficiency diagnosis or non-APDS immunodeficiency patients with disease pathophysiology that includes mTOR pathway activation, merits further investigation. For APDS1/2 patients, progress towards improving HCT outcomes may come from work related to tailoring serotherapy dosing, optimizing lymphodepletion over myeloablation, avoidance of post-HCT mTORi until there is confidence that residual host lymphocytes are eradicated, and optimizing the immune dysregulation pre-HCT as best possible.

In comparing the clinical manifestations pre-HCT in our cohort and in the cohort described by Coulter et al 2017,[3] in which 91% of patients did not proceed to HCT, patients in our cohort who all ultimately proceeded to HCT had a greater baseline frequency of enteropathy (57% vs. 25%), immune cytopenias (33% vs. 17%), hematologic malignancy (26% vs. 13%), suggesting that these manifestations may be particularly refractory to standard therapy and may justify sooner consideration of HCT once identified. Many patients in our cohort entered HCT with active, uncontrolled manifestations of immune dysregulation. Optimizing pre-HCT disease status with disease-attenuating agents such as mTORi, rituximab, and PI3K inhibitors, as well as moving patients to HCT before significant organ dysfunction develops, might reduce some of the struggles with graft stability and failure while also affording utilization of lower toxicity approaches to successful HCT. Baseline liver pathology was observed frequently, and SOS developed even after RIC. Poor pulmonary function pre-HCT was also frequent, and severe post-HCT respiratory complications such as acute respiratory distress syndrome or extracorporeal membrane oxygenation requirement were seen in 9% of patients. The long-term impact of these transplant-related organ toxicities on morbidity and longevity are of concern. Thus, the choice of conditioning intensity for these patients must be closely guided by underlying comorbidities; full lung function evaluations including spirometry and diffusion capacity of carbon monoxide whenever possible, in children as well as adults, along with a low threshold for detailed liver evaluation prior to HCT, may help inform these decisions.

The incidence of pre-HCT renal pathology and post-HCT renal complications was also notable in this cohort, with 2 cases of severe chronic kidney disease long-term. One of these patients developed post-HCT focal segmental glomerulosclerosis, a feature reported pre-HCT in other APDS patients, in the setting of full donor chimerism. It has been shown that hyperactivated PI3K-Akt signaling within kidney podocytes sensitizes them to injury and apoptosis[42] and has also been linked to renal tissue hyperproliferation and renal cell carcinoma, a complication observed post-HCT in one patient in this cohort.[43, 44] These findings suggest that APDS patients may remain at risk of renal complications post-HCT even if the hematopoietic system is fully donor and may merit closer observation in this regard, both due to possible increased vulnerability to renal insults early post-HCT and for chronic complications long term.

The minimum donor chimerism necessary for phenotype reversal remains to be defined, but it is notable that phenotype reversal has been observed in the setting of stable mixed chimerism in some patients. Regardless, given the risk of graft loss or instability, close monitoring of donor chimerism, including $CD3^+$ donor chimerism when possible, is necessary to identify declining donor chimerism early enough to intervene and should continue long term even after full donor chimerism is established, as illustrated by secondary GF and return of disease manifestations 5 years post-RT-MAC MUD HCT in a patient who ultimately required 3 HCTs.

Identifying the HCT approach associated with the most desirable immune reconstitution profile was not feasible based on the data available and given the heterogeneity of platforms, donors, and graft sources used in this cohort, along with the numerous DCIs administered for various indications. In terms of infectious complications, CMV and EBV requiring treatment, which are particularly problematic pre-HCT in APDS patients, occurred with similar frequency as would be expected in a general HCT recipient population.[45, 46] There was a subset of patients in this cohort with prolonged, profound lymphopenia and future studies should aim to characterize if such occurrences are related to the HCT approach/complications or might be inherent, non-hematopoietic disease features for a subset of APDS1/2 patients. Immune

reconstitution may be affected by serotherapy use, but serotherapy-free HCTs were few in this cohort, thus not providing ample comparison to serotherapy-containing approaches. Given the probable importance of serotherapy in the conditioning of these patients as well as the tendency for APDS1/2 patients to enter HCT with poor viral control and/or virus-associated malignancy, fine-tuning serotherapy exposure to the graft is future work of utmost importance when striving to improve immune reconstitution and avoid viral complications, while also optimally preventing GVHD.[47]

In conclusion, this large international investigation of HCT outcomes for APDS1/2 patients has yielded important insights into the breadth and severity of pre-HCT comorbidities, ongoing limitations in disease optimization pre-HCT, the incidence of major HCT complications, and key factors that may affect graft stability and loss. Use of mTOR inhibitors post-HCT appears to be detrimental to graft stability and should be avoided in the setting of clinically significant mixed or split donor chimerism. Longer detailed follow-up of graft stability, late toxicities, immune reconstitution, and phenotype reversal is needed to further inform the optimal timing of and approach to HCT for patients with APDS1/2.

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AUTHORSHIP CONTRIBUTIONS

J.A.K. designed the study with input from D.D., Z.N., and M.S.; D.D. and Z.N. managed the collection and cleaning of data that were contributed by all authors; D.D. and J.A.K. analyzed the data; D.D., Z.N., and J.A.K. wrote the manuscript with review, edits, and final manuscript approval from all authors.

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Table 1. Patient baseline characteristics

	Patients (n=57)
Demographics	
Male, n (%)	35 (61%)
Age at time of 1st HCT, yr, median (range)	13 (2-66)
Genetic defect[a], n (%)	
Known at time of 1st HCT	45 (79%)
Familial (confirmed)	10 (18%)
PIK3CD gain of function, heterozygous	43 (75%)
c.3061G>A, p.E1021K	33
Other	10[b]
PIK3RA splice site mutation, heterozygous	14 (25%)
c.1425+1 G>A	10
Other	4[b]
Clinical history and phenotype, n (%)	
Non-infectious lung pathology	38 (67%)
Gastrointestinal pathology	32 (57%)
History of immune cytopenias	19 (33%)
Hematological malignancy	15 (26%)
B cell lymphoma	13 (23%)[c,d]
Multiple myeloma	1 (2%)
Hepatosplenic $CD8^+$ T cell lymphoma	1 (2%)
Liver pathology	11 (19%)
Known nodular regenerative hyperplasia or portal hypertension	7 (12%)
Renal pathology	8 (14%)
Cardiac pathology	4 (7%)
Prior therapies, n (%)	
Immunoglobulin infusions	49 (86%)
mTOR inhibitor	28 (49%)
Rituximab	23 (40%)
Non-malignant indication (lymphoproliferation, autoimmunity)	15 (26%)
As part of multidrug chemotherapy for malignancy	8 (14%)
Splenectomy	10 (18%)
PI3K inhibitor	4 (7%)
Clinical status at HCT	
HCT-CI score at first HCT (n=48), median (range)[d]	2 (0-7)
Pediatric only (n=37), median (range)	2 (0-7)
Adult only (n=11), median (range)	4 (1-6)
HCT-CI score >=3, n (%)	21 (44%)
Pediatric only (n=37), HCT-CI score >=3, n (%)	12 (32%)
Adult only (n=11), HCT-CI score >=3, n (%)	9 (82%)
Karnofsky/Lansky performance status at first HCT (n=44), median (range)	90% (30%-100%)
Hematological malignancy status at HCT, n (%)	
In complete remission at HCT	10 (18%)
In partial remission or active at HCT	6 (11%)[e]
Hepatosplenomegaly, n (%)	29 (51%)
Active infection, n (%)	29 (51%)
Active immune cytopenias, n (%)	10 (18%)

Abbreviations: *HCT*, hematopoietic cell transplantation; *HCT-CI,* HCT comorbidity index; *mTOR*, mammalian target of rapamycin

[a]Pathogenicity was confirmed for all mutations.

[b]Other *PIK3CD* mutations: c.1573G>A, p.E525K (n=3); c.1002C>A, p.N334K (n=2); c.1246T>C, p.C416R (n=2); c.3074A>G, p.E1025G (n=1); c.1574A>C p.E525A (n=1); c.371 G>A, p.G124D (n=1). Other *PIK3RA* mutations: c.1425+1 G>T (n=2); n=1 each: c.1422_1 425+1 delCCAG, c.1425+1 G>C.

[c]B cell lymphoma details: diffuse large B cell lymphoma (n=7, one patient with 2 separate lymphomas; 5 EBV positive, 2 EBV negative); Hodgkin lymphoma (n=6; 2 EBV positive, 2 EBV negative, EBV data not available for n=2), marginal zone lymphoma (n=2; 1 EBV positive, 1 EBV negative).

[d]Lung function results, forced expiratory volume (FEV1) and diffusing capacity of carbon monoxide (DLCO), were available in 8 of 37 pediatric patients, FEV1 only in 17, DLCO only in 1, and not performed in 11, due to patient inability or center practices. Thus, HCT-CI scores in pediatric patients may be underestimated. Of 11 adults, FEV1 and DLCO were available for 9, and FEV1 only for 2.

[e]One patient had two B cell lymphomas prior to HCT, EBV+ nodular sclerosing classical Hodgkin lymphoma in CR1 at time of HCT, and EBV+ marginal zone lymphoma active and untreated prior to HCT.

Table 2a. Donor and graft characteristics

	HCTs (n=66)
Graft source, n (%)	
Bone marrow	32 (48%)
PBSC	31 (47%)
Single umbilical cord	3 (5%)
Graft dose by graft type, TNC x10^8/kg, median (range)	
Bone marrow, unmanipulated (n=29)	3.47 (0.86-9.67)
PBSC, unmanipulated (n=21)	12.1 (4-40.7)
PBSC, manipulated (n=7)	8 (0.092-13.3)
Single umbilical cord (n=3)	0.499 (0.34-0.5)
Donor source, n (%)	
Unrelated (non-cord)	41 (62%)
HLA-10/10	29 (44%)
HLA-8/8	36 (55%)
Other: HLA-7/8 (n=3), HLA-5/8 (n=1), HLA 6/6 (n=1)	5 (8%)
HLA-haploidentical	15 (23%)
HLA-matched sibling	7 (11%)
Female donor into male recipient, n (%)[a]	**16 (28%)**
Donor age, yr, median (range)[a]	**27 (4-58)**
ABO, n (%)[a]	
Matched	29 (50%)
Minor mismatch	15 (26%)
Major mismatch	12 (21%)
Major and minor mismatch	2 (3%)
CMV serostatus D/R, n (%)[a]	
D+/R+	23 (40%)
D+/R unknown	13 (22%)
D+/R-	1 (2%)
D-/R+	8 (14%)
D-/R-	7 (12%)
D-/R unknown	6 (10%)

Table 2b. Transplant platform characteristics, by donor type

	Total HCTs (n=66)	MSD HCTs (n=7)	8/8 MUD HCTs (n=36)	Haplo HCTs (n=15)	Other HCTs (n=8)
Conditioning intensity, n (%)					
Myeloablative conditioning	24 (36%)	7 (100%)	11 (31%)	5 (33%)	1 (13%)
Reduced-toxicity myeloablative conditioning	6 (9%)	0	6 (17%)	0	0
Reduced-intensity conditioning	35 (53%)	0	19 (53%)	9 (60%)	7 (88%)
Non-myeloablative conditioning	1 (2%)	0	0	1 (7%)	0
Serotherapy use, n (%)	**55 (83%)**	**5 (71%)**	**34 (84%)**	**11 (73%)**	**5 (63%)**
Anti-thymocyte globulin	33 (50%)	4 (57%)	18 (50%)	8 (53%)	3 (38%)
Rabbit	29 (44%)	4 (57%)	15 (42%)	7 (47%)	3 (38%)
Horse	4 (6%)	0	3 (8%)	1 (7%)	0
Alemtuzumab	22 (33%)	1 (14%)	16 (44%)	3 (20%)	2 (25%)
Proximal timing (administered day -8 or closer to HCT)	15 (23%)	1 (14%)	12 (33%)	0	2 (25%)
Intermediate timing (administered between days -16 and -9)	7 (11%)	0	4 (11%)	3 (20%)	0
Total body irradiation (total dose 2 to 4 Gy), n (%)	**12 (18%)**	**0**	**6 (17%)**	**3 (20%)**	**3 (38%)**
Graft-versus-host disease prophylaxis, n (%)					
Calcineurin-inhibitor-based	48 (73%)	7 (100%)	29 (81%)	5 (3%)	7 (88%)
Post-transplantation cyclophosphamide-based	13 (20%)	0	5 (14%)	8 (53%)	0
Graft manipulation	8 (13%)	0	4 (11%)	3 (20%)	1 (13%)
Alpha/beta T cell/CD19$^+$ depletion, with or without CD45RA$^-$ addback	6 (9%)	0	3 (8%)	2 (13%)	1 (13%)
Alpha/beta T cell depletion	1 (2%)	0	0	1 (7%)	0
CD34$^+$ positive selection	1 (2%)	0	1 (3%)	0	0
No pharmacologic prophylaxis apart from serotherapy	3 (5%)	0	0	2 (13%)	1 (13%)
Other[b]/incomplete information	2 (3%)	0	2 (6%)	0	0

Abbreviations: *CMV*, cytomegalovirus; *D/R*, donor/recipient; *HLA*, human leukocyte antigen; *HCT*, hematopoietic cell transplantation; *PBSC*, peripheral blood stem cells; *TNC*, total nucleated cells.

[a]Number HCTs for which data was available: donor sex, ABO compatibility, and CMV serostatus (n=58); donor age (n=53).

[b]In addition to rabbit anti-thymocyte globulin and graft manipulation, patient received abatacept and mycophenolate mofetil early post-HCT, followed by methotrexate.

Table 3. Engraftment and subsequent unplanned cell infusions.

	1st HCT, patients (n=57)	**2nd HCT, patients (n=8)**	**3rd HCT, patients (n=1)**
Engraftment, n (%)			
Primary graft failure	2 (4%)	0	0
Secondary graft failure	7 (12%)	1 (13%)	0
Unstable chimerism or threatened graft failure (not progressing to graft failure)	4 (7%)	2 (25%)	0
Poor graft function	9 (16%)	3 (38%)	0
Subsequent unplanned cell infusion, n patients (%)[a]	18 (32%)	4 (50%)	0
Donor lymphocyte infusion[b]	7 (12%)	2 (25%)	0
Repeat HCT[c]	8 (14%)	1 (13%)	0
Peripheral blood stem cell boost[d]	5 (9%)	1 (13%)	0

[a]A total of 43 subsequent unplanned cell infusions were administered.

[b]A total of 24 unplanned donor lymphocyte infusions were administered, for mixed chimerism (n=18), viral infection (n=2), lymphoma relapse (n=2), poor graft function (n=1), promoting immune reconstitution (n=1).

[c]Indications for repeat HCT included graft failure (n=6), mixed chimerism (n=2), and lymphoma relapse (n=1).

[d]A total of 10 stem cell boosts were administered, for poor graft function (n=9) and to promote immune reconstitution (n=1, included CD3+ add back).

Table 4a. Outcomes for all patients

Outcome	Patients (n=57)
Transplant-related mortality, n (%)	**8 (14%)**
Infection[a]	6 (11%)
Organ toxicity (regimen-related)	2 (4%)
Acute GVHD, n (%)	**22 (39%)**
Grade 2-4	13 (23%)
Grade 3-4	4 (7%)
Chronic GVHD[b]	**9 (16%)**
Mild	3 (11%)
Moderate	1 (2%)
Severe	2 (4%)
Organ toxicities	
Renal failure requiring dialysis[c]	6 (11%)
Sinusoidal obstructive syndrome[d]	3 (5%)
Congestive heart failure	3 (5%)
ARDS	3 (5%)
Respiratory failure requiring ECMO[d]	2 (4%)
DAH, IPS, BO, or COP	0
Infectious complications	
CMV infection requiring treatment; CMV disease	26 (46%); 4 (7%)
EBV in blood requiring therapy; EBV-PTLD	6 (11%); 3 (5%)
Adenoviremia requiring treatment; adenovirus with organ involvement	4 (7%); 1 (2%)
HHV-6 in blood requiring treatment; HHV-6 encephalitis	5 (9%); 0
BK virus-associated hemorrhagic cystitis; biopsy-proven BK nephropathy	10 (18%); 1 (2%)
Lower respiratory viral infection other than CMV or adenovirus	8 (14%)
HSV requiring treatment	6 (11%)
VZV requiring treatment	3 (5%)
Bacteremia, with or without sepsis; sepsis	19 (33%); 9 (16%)
Other significant bacterial infection	10 (18%)
Fungal infection requiring systemic treatment	3 (5%)
Pneumocystis jiroveci pneumonia	1 (2%)
Toxoplasmosis reactivation	0

Abbreviations: *ARDS*, acute respiratory distress syndrome; *BO,* bronchiolitis obliterans; *CMV,* cytomegalovirus; *COP,* cryptogenic organizing pneumonia; *DAH,* diffuse alveolar hemorrhage; *EBV,* Epstein-Barr virus; *ECMO*, extracorporeal membranous oxygenation; *GVHD*, graft-versus-host disease; *HHV*-6, human herpesvirus-6; *HSV,* herpes simplex virus; *IPS,* idiopathic pneumonia syndrome; *PTLD,* post-transplantation lymphoproliferative disease; *VZV*, varicella zoster virus.

[a]Attributed to *Pseudomonas aeruginosa* (n=3, at days +6, +238, +340), *Rhizomucor pusillus* (n=1, day +17), sepsis in asplenic patient (n=1, day +663), and CMV disease (n=1, day +75). Only the last patient had active GVHD (grade 2, skin only) requiring systemic corticosteroids, diagnosed a week before death; one other patient had grade 2, skin only GVHD which developed following donor lymphocyte infusion for lymphoma relapse, treated with calcineurin inhibitor alone, but died of multidrug resistant *Pseudomonas*.

[b]Chronic skin GVHD without available data on severity reported for 3 patients. One patient had probable ocular-only chronic GVHD, not diagnostic of GVHD per 2014 consensus criteria and not included above.

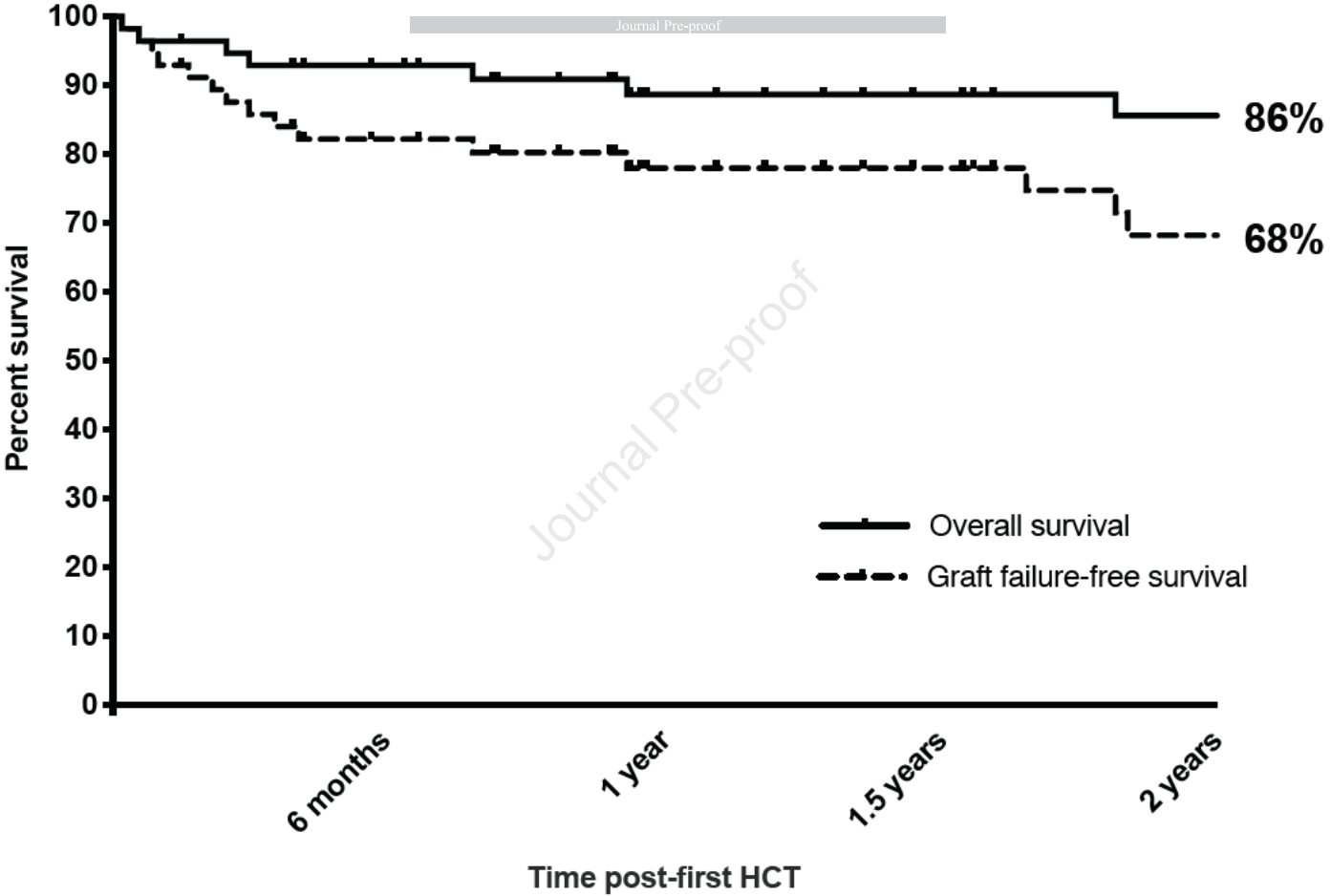
[c]Occurred in the setting of severe infection (n=5), sinusoidal obstructive syndrome and thrombotic microangiopathy (n=1). Three patients had significant known pre-existing renal pathology. Two patients had concurrent respiratory failure requiring ECMO; outcomes included TRM (n=4) and chronic kidney disease (n=2, focal segmental glomerulosclerosis on immunosuppression; BK-associated nephropathy requiring hemodialysis).

[d]Developed following RIC in all 3 patients, 2 of whom had known prior known liver pathology (n=2). The remaining patient, without prior known liver pathology, received 16mg/kg total of busulfan, targeting area under the curve of 60mg*h/L.

Table 4b. Outcomes of engrafted survivors

Outcome	Patients (n=48)
Alive and well with phenotype reversal, n (%)	**41 (85%)**
Full donor chimerism (>95%)[a]	33 (69%)
Mixed $CD3^+$ donor chimerism only (<95%)[b]	3 (6%)
Mixed donor chimerism in both compartments (<95%)[c]	3 (6%)
Other[d]	2 (6%)
Alive with phenotype reversal but significant ongoing complications[e], n (%)	**4 (8%)**
Other	**3 (6%)**
Partial phenotype reversal, mixed donor chimerism[f]	2 (4%)
Too early to evaluate phenotype reversal (<100 days post-HCT)	1 (2%)

[a]Whole blood or myeloid donor chimerism >95%; $CD3^+$ chimerism, if available (n=14), was >95%.
[b]Whole blood or myeloid donor chimerism >95%; $CD3^+$ chimerism <95% (9.2%, 40%, 93%).
[c]Whole blood or myeloid donor chimerism <95% (range 52-82%) and $CD3^+$ chimerism <95% (range 67.5-84%).
[d]Whole blood donor chimerism <95% (n=1, 94%) and full $CD3^+$ donor chimerism but mixed myeloid and whole blood donor chimerism (n=1, 96%, 86%, 90% respectively).
[e]Ongoing complications include GVHD (n=2), chronic kidney disease (n=2). All have 100% donor chimerism.
[f]One patient has resolution of recurrent respiratory infections and enteropathy with negative EBV, CMV, and adenovirus in blood, but recent ocular HSV and VZV despite prophylaxis 2 years post-HCT; last donor chimerism 35% whole blood, 26% myeloid, 34% $CD3^+$, 17% $CD19^+$. The other patient has improvement of disseminated *Mycoplasma orale* infection but continued immune thrombocytopenia and hypogammaglobulinemia 2.8 years post-HCT; last donor chimerism 85% myeloid, 58% $CD3^+$, 99% $CD19^+$, 82% natural killer.



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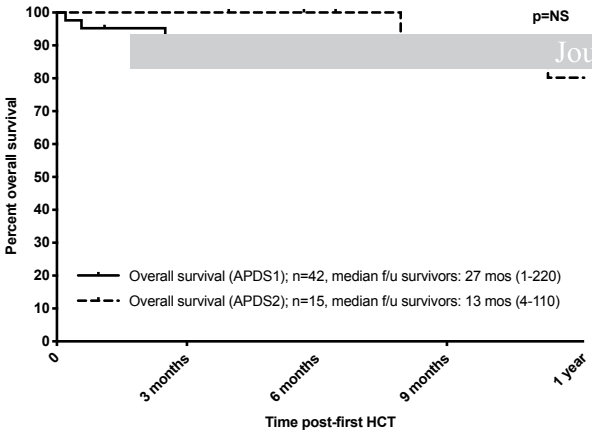

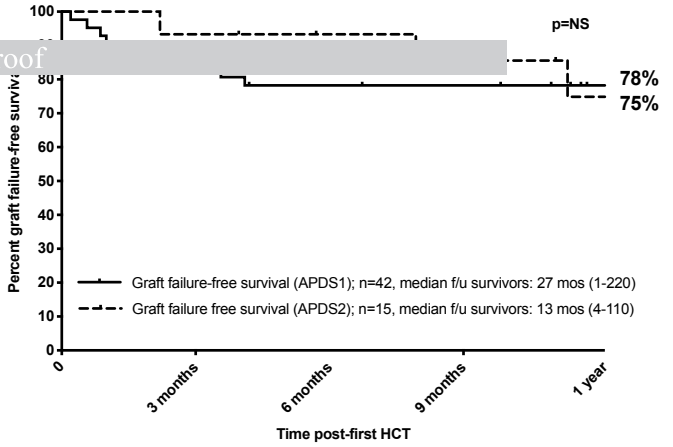

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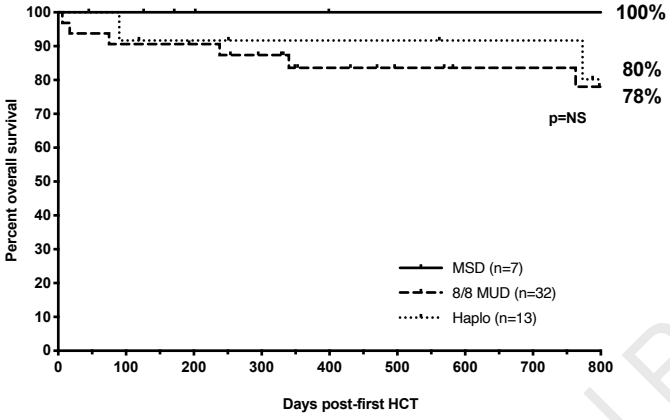

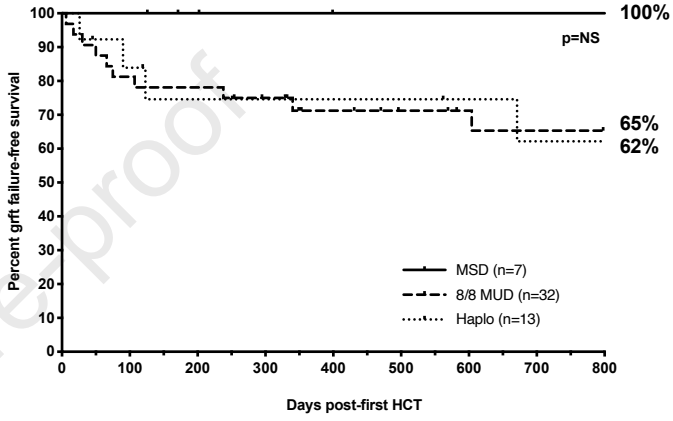

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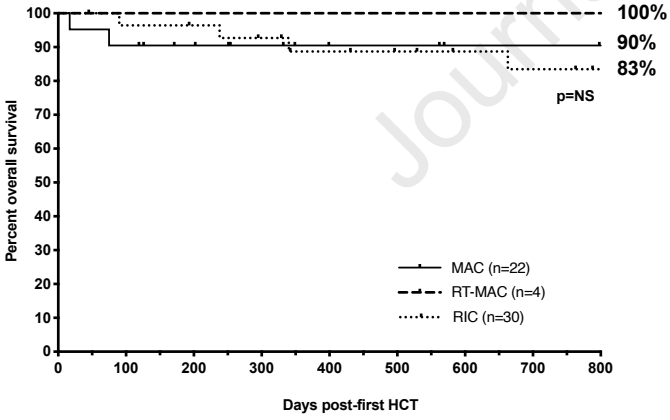

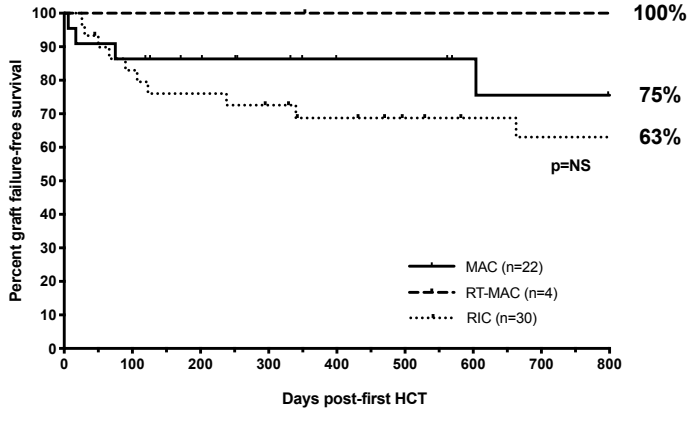

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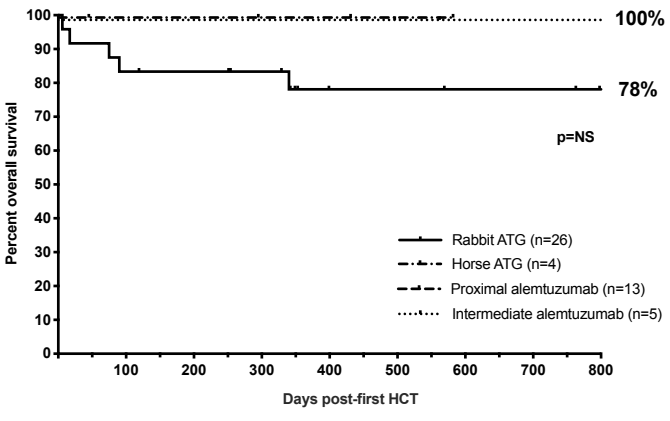

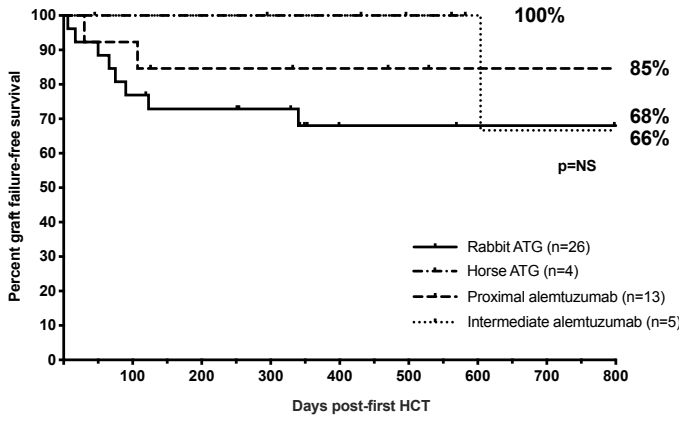

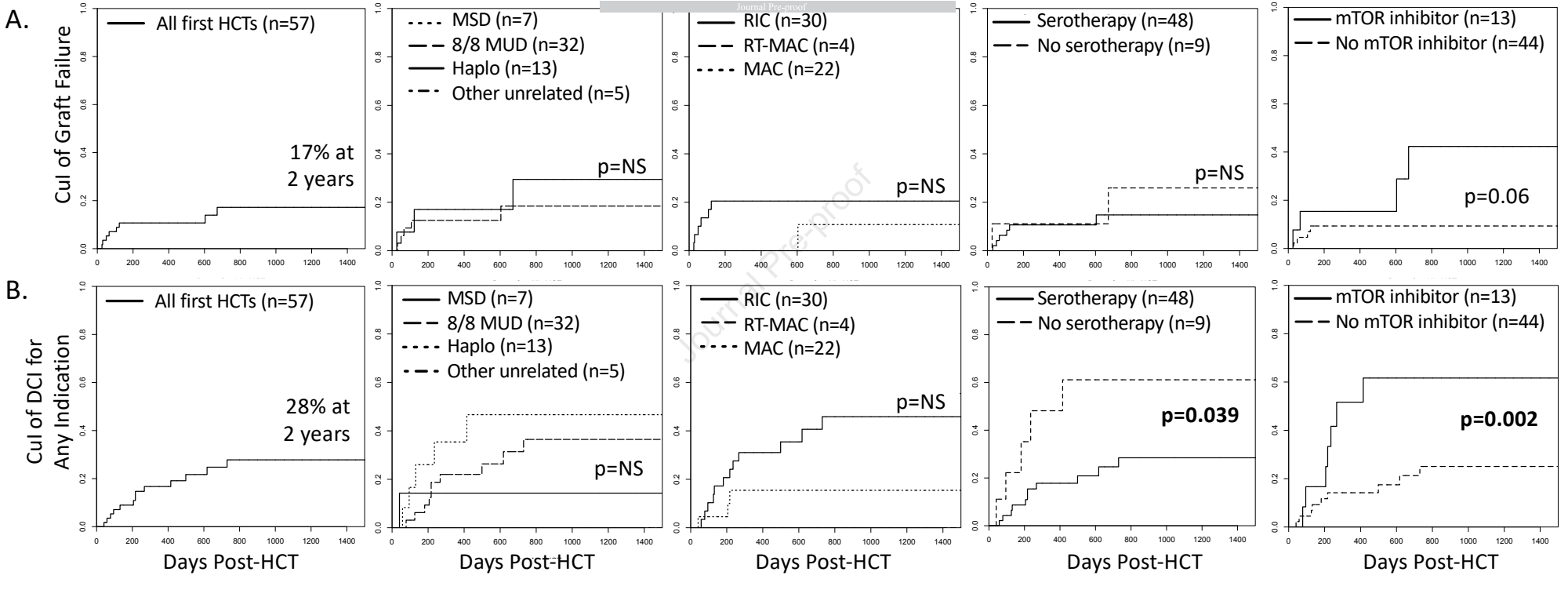

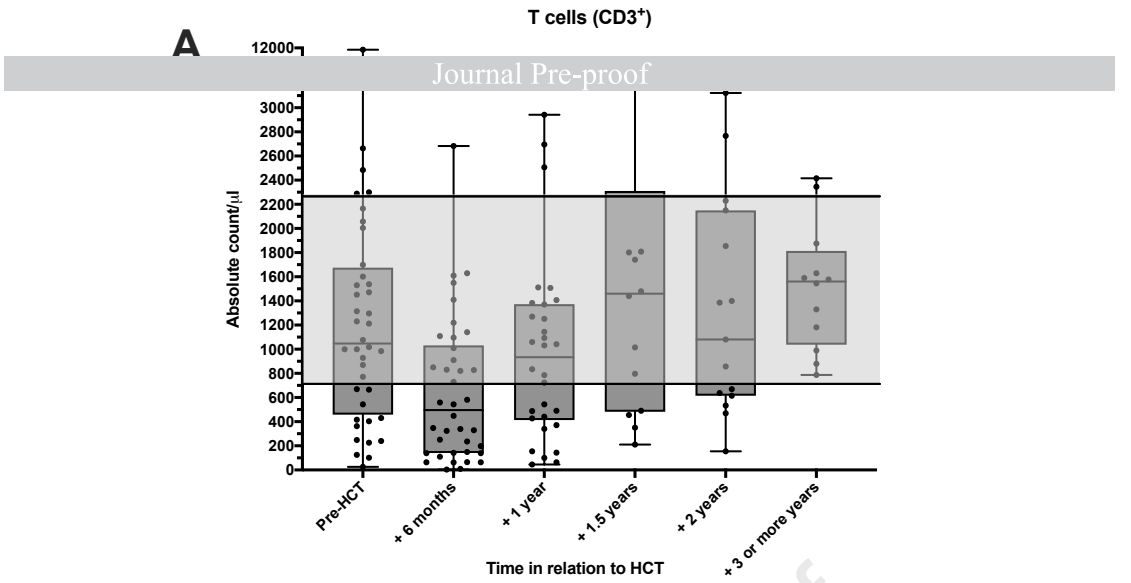

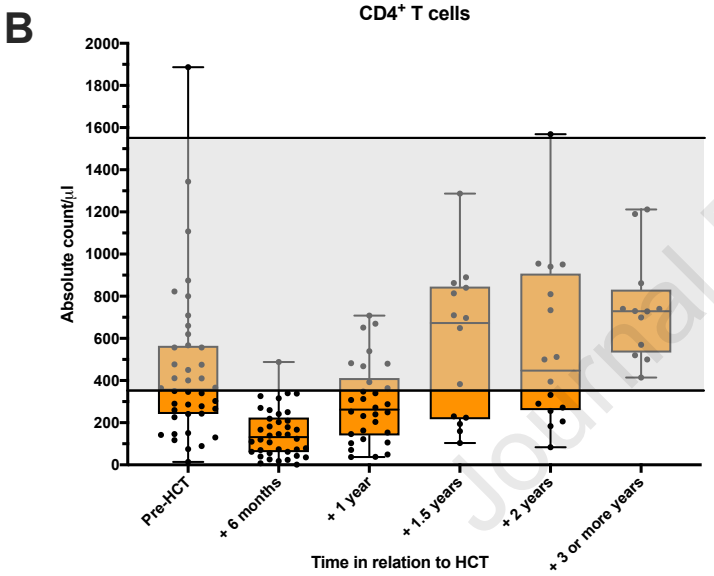

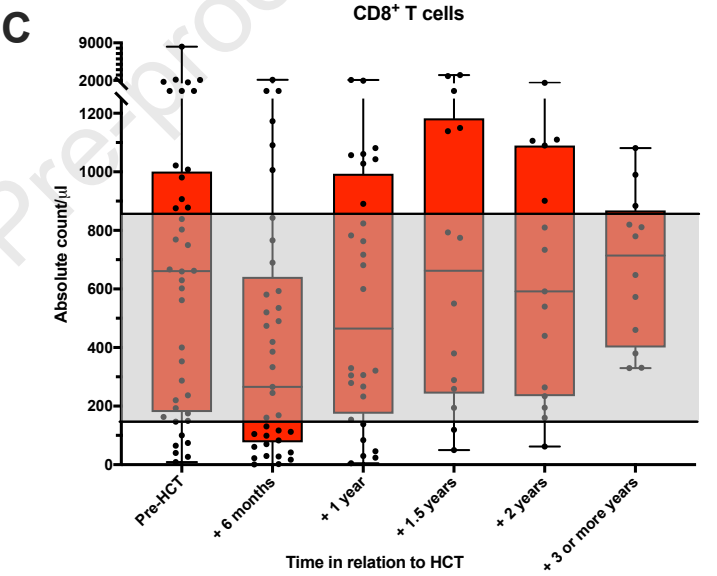

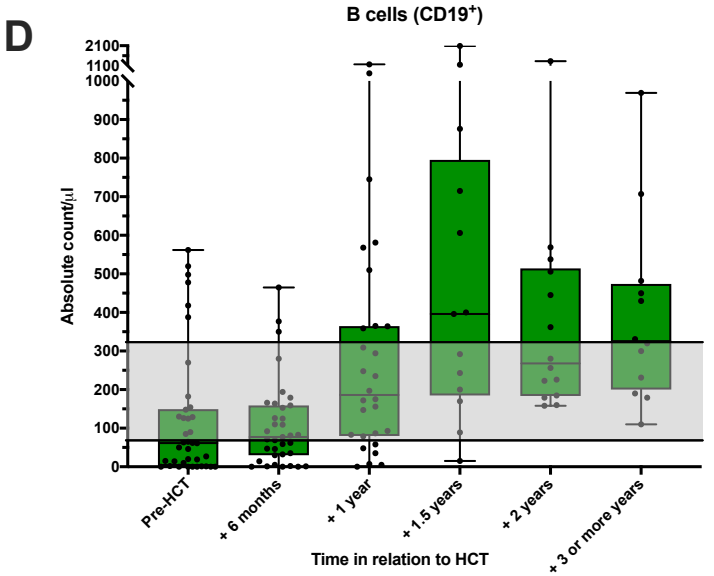

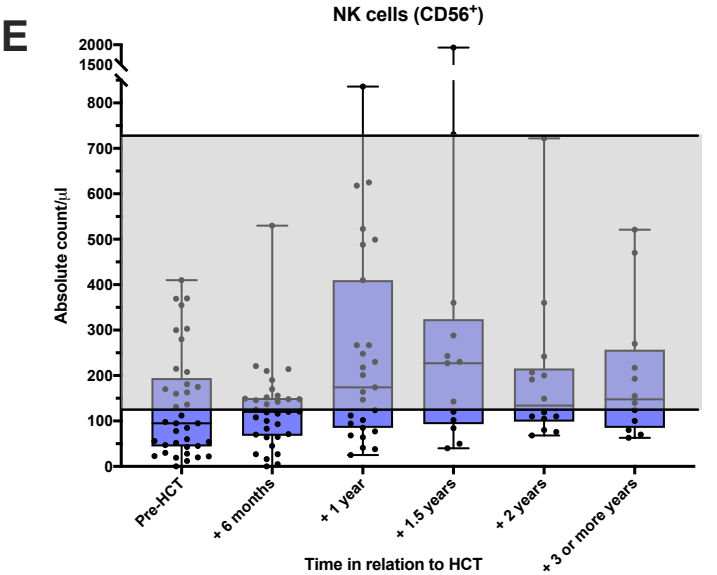

Figure 1. Kaplan-Meier survival curves depicting overall survival and graft failure-free survival for all patients, n=57, with median follow up 27 months overall by the reverse Kaplan-Meier method and 26.3 months (range 1.5-220.6) from first HCT in survivors.

Figure 2. Kaplan-Meier survival curves depicting overall survival and graft failure-free survival by underlying diagnosis (A), donor type (B; smallest subgroup of mismatched unrelated donor and cord excluded, n=5), conditioning intensity (C; smallest subgroup of nonmyeloablative/immunosuppression only conditioning excluded, n=1), or serotherapy choice (D; of note, follow up is shorter for smaller subgroups of horse antithymocyte globulin (ATG) and intermediate timing alemtuzumab).

Figure 3. Cumulative incidence (CuI) of graft failure after first HCT (A) and subsequent unplanned donor cell infusion (DCI) for any indication after first HCT (B), overall and by donor type, conditioning intensity (excludes nonmyeloablative immunosuppression-only conditioning, n=1), serotherapy use during conditioning, and mTOR inhibitor use within the first year after HCT. No graft failure was observed with MSD or other unrelated donor (mismatched, n=4; cord, n=1). One patient had graft failure 1862 days post-RT-MAC-HCT requiring re-transplantation (not depicted).

Figure 4. Lymphocyte subset counts pre-HCT and reconstitution post-HCT in total T cells (A), CD4+ T cells (B), CD8+ T cells (C), B cells (D), and NK cells (E). Only patients with data for at least one post-HCT timepoint are included. One patient is represented twice, at time of 1st HCT and at time of subsequent HCT 5 years later.