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ORIGINAL ARTICLE

Reduced-Intensity/Reduced-Toxicity Conditioning Approaches Are Tolerated in XIAP Deficiency but Patients Fare Poorly with Acute GVHD

Danielle E. Arnold[1] · Rofida Nofal[2] · Connor Wakefield[3] · Kai Lehmberg[4] · Katharina Wustrau[4] · Michael H. Albert[5] · Emma C. Morris[6] · Jennifer R. Heimall[7] · Nancy J. Bunin[8] · Ashish Kumar[1] · Michael B. Jordan[1] · Theresa Cole[9] · Sharon Choo[9] · Tim Brettig[9] · Carsten Speckmann[10] · Stephan Ehl[10] · Malgorzata Salamonowicz[11] · Justin Wahlstrom[12] · Kanchan Rao[13] · Claire Booth[14] · Austen Worth[14] · Rebecca A. Marsh[1]

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Abstract

X-linked inhibitor of apoptosis (XIAP) deficiency is an inherited primary immunodeficiency characterized by chronic inflammasome overactivity and associated with hemophagocytic lymphohistiocytosis (HLH) and inflammatory bowel disease (IBD). Allogeneic hematopoietic cell transplantation (HCT) with fully myeloablative conditioning may be curative but has been associated with poor outcomes. Reports of reduced-intensity conditioning (RIC) and reduced-toxicity conditioning (RTC) regimens suggest these approaches are well tolerated, but outcomes are not well established. Retrospective data were collected from an international cohort of 40 patients with XIAP deficiency who underwent HCT with RIC or RTC. Thirty-three (83%) patients had a history of HLH, and thirteen (33%) patients had IBD. Median age at HCT was 6.5 years. Grafts were from HLA-matched ($n=30$, 75%) and HLA-mismatched ($n=10$, 25%) donors. There were no cases of primary graft failure. Two (5%) patients experienced secondary graft failure, and three (8%) patients ultimately received a second HCT. Nine (23%) patients developed grade II–IV acute GVHD, and 3 (8%) developed extensive chronic GVHD. The estimated 2-year overall and event-free survival rates were 74% (CI 55–86%) and 64% (CI 46–77%), respectively. Recipient and donor HLA mismatch and grade II–IV acute GVHD were negatively associated with survival on multivariate analysis with hazard ratios of 5.8 (CI 1.5–23.3, $p=0.01$) and 8.2 (CI 2.1–32.7, $p<0.01$), respectively. These data suggest that XIAP patients tolerate RIC and RTC with survival rates similar to HCT of other genetic HLH disorders. Every effort should be made to prevent acute GVHD in XIAP-deficient patients who undergo allogeneic HCT.

Keywords XIAP deficiency · Hemophagocytic lymphohistiocytosis · Hematopoietic cell transplantation · Reduced-intensity conditioning · Graft-versus-host disease · Austen Worth and Rebecca A. Marsh contributed equally to this work

Introduction

X-linked inhibitor of apoptosis (XIAP) deficiency is an X-linked inborn error of immunity that was discovered in 2006. [1] XIAP has several critical functions in the regulation of cell survival and inflammation. XIAP inhibits apoptosis by direct interaction and inhibition of caspase-3, caspase-7, and caspase-9. [2–5] XIAP facilitates nucleotide-binding oligomerization domain-containing (NOD)-1 and -2 receptor-mediated nuclear factor-κB signaling. [6–10] XIAP is also a critical regulator of receptor-interacting protein kinase 1 (RIPK1) mediated TNF receptor signaling, and deficiency of XIAP results in dysregulated inflammasome function. [11–15] Patients with XIAP deficiency have evidence of chronic inflammasome activity even during periods of wellness, as demonstrated by chronically elevated blood levels of IL-18. [16].

Several inflammatory disease manifestations develop in patients with XIAP deficiency, including hemophagocytic lymphohistiocytosis (HLH), incomplete HLH-like manifestations, recurrent fevers, arthritis, uveitis, and inflammatory bowel disease. [1, 17–23] These manifestations may be largely driven by dysregulated TNF receptor signaling and inflammasome activity with overproduction of IL-1beta and IL-18, and resultant propensity to inflammation. A wide range of disease severity has been observed, and

✉ Rebecca A. Marsh
Rebecca.Marsh@cchmc.org

Extended author information available on the last page of the article

some patients with severe disease are optimally treated with allogeneic hematopoietic cell transplantation (HCT). Unfortunately, allogeneic HCT was initially reported to be associated with a high risk of mortality in patients with XIAP deficiency. An early survey of 19 patients in 2013 observed survival of only 14% in patients who were treated with fully myeloablative conditioning regimens ($N=7$). [24] Regimen-related toxicities including hepatic veno-occlusive disease and pulmonary hemorrhage were common causes of death, suggesting that deficiency of XIAP may render patient tissues more susceptible to chemotherapeutic agents.

Reduced-intensity conditioning (RIC) and reduced-toxicity conditioning (RTC) regimens have been used more often in recent years due to increased survival in genetic HLH disorders. [25–28] Survival following these approaches in two series of patients with XIAP deficiency ranged from 55 to 90%. [24, 29].

Despite the small reports of better outcomes with RIC and RTC regimens, larger analyses are needed to more accurately estimate patient survival. Additionally, studies are needed to assess whether patients with XIAP deficiency are at increased risk of transplant-associated morbidity and mortality due to an increased risk of inflammatory complications, such as graft-versus-host disease (GVHD), as non-hematopoietic cell–derived tissues remain XIAP-deficient following allogeneic HCT. Notably, two recent murine studies have reported increased severity of GVHD and increased mortality among transplanted XIAP-deficient recipient mice, likely related to lingering recipient XIAP-deficient cell dysregulation of inflammation and cell death. [30, 31].

In an effort to more accurately estimate the survival of RIC and RTC approaches in a larger cohort of patients with XIAP deficiency, and to understand the impact of GVHD on outcomes, we conducted a retrospective study of RIC and RTC allogeneic HCT outcomes at several collaborating centers.

Methods

Patients

Institutional review board approval was obtained for this study and was in accordance with the guidelines in the 1964 Declaration of Helsinki and its later amendments. Patients with XIAP deficiency who had undergone allogeneic HCT were identified by request to national and international sites known to the investigators to have performed allogeneic HCT for XIAP deficiency. To be included, patients were required to have a confirmed deleterious hemizygous mutation in *XIAP* ($n=39$) except for 1 additional included patient who had absence of XIAP protein expression (confirmed with multiple samples) but for whom a genetic mutation was not found. Deidentified data were collected by using a spreadsheet questionnaire filled out by investigators at each institution. Of note, 8 patients were included in a previous report on transplant outcomes for XIAP deficiency by Marsh et al. (Supplemental Table 1). [24].

XIAP Protein Expression

Lymphocyte XIAP protein expression was performed for the majority of patients using either Western blot analysis or intracellular flow cytometric analysis with standard methods.

Transplant Characteristics

Routine HCT data was collected, including donor relation, human leukocyte antigen (HLA) match revealed by high-resolution HLA typing for HLA-A, -B, -C, and -DR, with or without HLA-DQ, graft source, conditioning regimen, and acute GVHD prophylaxis. Myeloablative and reduced-intensity conditioning regimen intensities were classified using CIBMTR workshop definitions. [32] Myeloablative conditioning regimens (MAC) were defined as regimens that contained an alkylating agent at a dose that would be expected to not allow autologous bone marrow recovery. Reduced-intensity conditioning (RIC) regimens were those regimens that did not meet the criteria for MAC. Busulfan- and treosulfan-containing regimens which contained myeloablative but reduced dosing in conjunction with fludarabine were grouped as reduced-toxicity regimens. HLA match and relationship categories included matched related donor (MRD), matched unrelated donor (MUD), mismatched unrelated donor (MMUD), and haploidentical donor.

Transplant Outcomes

Neutrophil recovery was defined as the first of 3 consecutive days with a peripheral blood absolute neutrophil count $\geq 0.5\times10^9$/L, and platelet recovery was defined as the first day with a platelet count $\geq 20\times10^9$/L without transfusion support for 7 consecutive days. Results of clinically performed chimerism studies were collected. Mixed chimerism was defined as detection of $<95\%$ donor chimerism in whole blood. Primary graft failure was defined as lack of neutrophil recovery by day + 42 or lack of donor chimerism $>5\%$ by day + 42, and secondary graft failure was defined as loss of donor cells with detection of $<5\%$ donor chimerism in whole blood after initial neutrophil recovery and beyond day + 42. One patient was classified as having delayed neutrophil recovery, as they met the definition of neutrophil recovery on day +60 after having received etoposide and other myelotoxic drugs post-HCT. Data regarding HCT complications and survival were also collected. Viral reactivations were included if they were considered clinically significant by

the treating institution, as defined by either the instigation of antiviral or cellular therapy or the presence of associated clinical manifestations. Acute GVHD (aGVHD) was graded based on consensus criteria. [33] Chronic GVHD (cGVHD) was reported as limited or extensive. [34].

Statistical Analysis

Binary and categorical variables were summarized by frequency (%), and continuous variables were summarized by median with range. Categorical data were compared using the chi-squared test. Overall and event-free survival were estimated using the Kaplan–Meier method, and groups were compared using the log-rank test. An event was defined as primary graft failure, secondary graft failure, receipt of a subsequent transplant, or death. Patients who underwent second transplant were censored at the time of second transplant for analysis of overall survival. Multivariate analyses were performed using Cox proportional hazards regression modeling with stepwise selection of covariates. The following variables were considered: age at HCT, patient history of HLH, recipient and donor HLA match, conditioning regimen intensity, and grade II–IV acute GVHD. A p value of < 0.05 was considered statistically significant.

Results

Patient Characteristics

Forty patients submitted by 10 participating centers from the USA, Germany, the UK, Poland, and Australia were included. Patient baseline characteristics are listed in Table 1. Thirty-nine (98%) patients were male, and there was one (3%) female patient with a hemizygous *XIAP* mutation with heavily skewed lyonization (0–9% of lymphocytes with normal XIAP expression) and clinical phenotype consistent with XIAP deficiency. Median age was 2.9 years (range 0–18) at the time of initial presentation and 5.5 years (range 0–24) at the time of diagnosis of XIAP deficiency. Thirty-nine (98%) patients had a confirmed hemizygous mutation in *XIAP* (Supplemental Table 1), and one patient lacked an observed mutation but had absent XIAP expression confirmed with multiple blood samples. XIAP protein expression was evaluated for 33 patients; XIAP protein expression was decreased in 9 (23%), absent in 22 (55%), bimodal in the 1 female patient (3%), and normal in 1 (3%) (Supplemental Table 1). The patient with normal XIAP protein expression had a nonsense mutation and clinical phenotype consistent with XIAP deficiency and, as such, expressed protein was presumed to be nonfunctional.

Table 1 Patient characteristics and clinical phenotype

Characteristic	$N = 40$
Male, *n* (%)	39 (97.5)
Age at presentation (yr), median (range)	2.9 (0–18)
Age at diagnosis (yr), median (range)	5.5 (0–24)
Disease manifestations, *n* (%)	
HLH	33 (82.5)
EBV-related HLH	14 (35)
Non-EBV-related HLH	24 (60)
CNS HLH	5 (12.5)
Recurrent fevers/cytopenia [a]	3 (7.5)
Inflammatory bowel disease	13 (32.5)
Hypogammaglobulinemia	14 (35)
HLH treatment, *n* (%)	
Dex + etoposide + CSA ± rituximab	14 (42.4)
Dex + etoposide	4 (12.1)
Pred/methylpred + CSA	1 (3.0)
Dex/pred/methylpred	12 (36)
Intrathecal MTX ± steroids	5 (15.2)
Alemtuzumab	2 (6.1)
No treatment [b]	1 (3.0)
HLH status at the time of HCT, *n* (%)	
Active	3 (9.1)
Partial remission	2 (6.1)
Remission	28 (84.8)

HLH hemophagocytic lymphohistiocytosis, *EBV* Epstein-Barr virus, *CNS* central nervous system, *dex* dexamethasone, *CSA* cyclosporine, *pred* prednisone, *methylpred* methylprednisolone, *MTX* methotrexate, *HCT* hematopoietic cell transplantation

[a]These 3 patients had a history of recurrent fevers/cytopenia but never met criteria for diagnosis of HLH

[b]This patient did not receive HLH-specific therapy but was on immunosuppressive therapy for treatment of inflammatory bowel disease

Clinical Phenotype and Pre-HCT Complications

Thirty-three of the forty patients (83%) had a history of HLH (Table 1 and Supplemental Table 2). Most (18/33 or 55%) patients received treatment with dexamethasone and etoposide with or without cyclosporine, and one (3%) patient received prednisone or methylprednisolone with cyclosporine. Twelve (36%) patients received steroids (dexamethasone, prednisone, and/or methylprednisolone) without etoposide or cyclosporine. Other therapies included rituximab ($n = 3$, 9%), alemtuzumab ($n = 2$, 6%), 6-mercaptopurine ($n = 1$, 3%), mycophenolate mofetil ($n = 1$, 3%), and sirolimus ($n = 1$, 3%). Five of the 33 (15%) patients with HLH had central nervous system involvement, all of whom received intrathecal methotrexate with or without intrathecal steroids. HLH was reported to be either active or in partial remission in 5 (15% of patients with HLH and 13% overall) patients at the time of HCT. Of note, 3 of the 40 (8%)

patients had a history of recurrent fevers and/or cytopenia without ever meeting diagnostic criteria for HLH.

In addition to HLH, IBD and hypogammaglobulinemia were common (Table 1 and Supplemental Table 2). Thirteen (33%) patients were diagnosed with IBD, and 14 (35%) had a history of hypogammaglobulinemia. One patient was diagnosed in the setting of an older brother with XIAP deficiency. He is the only patient that did not have a history of HLH, HLH-like illness, or IBD pre-HCT. Recurrent infections were common in the setting of HLH and IBD treated with chronic immunosuppression, but recurrent infections were not the disease-defining illness in any cases.

Transplant Procedures

Patients were transplanted between 2006 and 2016 (median year 2014). Transplant characteristics are shown in Table 2 and Supplemental Table 3. The median age at HCT was 6.5 years (range 0.45–27). Seven patients were adults (18 years of age or older) at the time of transplantation. Donor sources were matched related in 10 (25%), matched unrelated in 20 (50%), mismatched unrelated in 7 (18%), and haploidentical in 3 (8%) cases. Of note, one patient received a graft from a matched female sibling donor who was a carrier of XIAP deficiency. Bone marrow was used in 25 (63%), T-replete peripheral blood stem cells (PBSC) in 11 (28%), and T-deplete PBSC in 4 (10%) transplants. Most (24/40 or 60%) patients received alemtuzumab, fludarabine, and melphalan with or without thiotepa. Five (13%) patients received anti-CD45 antibody, alemtuzumab, fludarabine, and cyclophosphamide. The remaining eleven (28%) patients received treosulfan or busulfan plus fludarabine-based conditioning. Most ($n=36$, 90%) patients received calcineurin inhibitor-based GVHD prophylaxis, and 2 (5%) patients received sirolimus plus mycophenolate mofetil. One (3%) patient received post-transplant cyclophosphamide with calcineurin inhibitor and mycophenolate mofetil. Of the 4 patients who received T-deplete PBSCs, 2 (5%) received a calcineurin inhibitor plus MMF, 1 (3%) received mycophenolate mofetil monotherapy, and 1 (3%) did not receive additional GVHD prophylaxis.

Table 2 Transplant characteristics

Characteristic	$N=40$
Age at HCT (yr), median (range)	6.5 (0.45–27)
Donor type, n (%)	
MRD	10 (25.0)
MUD	20 (50.0)
MMUD	7 (17.5)
Haploidentical	3 (7.5)
Graft source, n (%)	
Bone marrow	25 (62.5)
T-replete PBSC	11 (27.5)
T-deplete PBSC	4 (10.0)
Cell dose, median (range)	
Total nucleated cells × 10^8/kg	7.0 (2.0–33.5)
CD34 + cells × 10^6/kg	5.9 (1.6–40.6)
CD3 + cells × 10^7/kg	3.7 (0.3–15.9)
Conditioning regimen, *n* (%)	
Flu/mel ± TT ± alem or ATG	24 (60.0)
Anti-CD45/flu/Cy + alem	5 (12.5)
Treo or bu/flu ± TT ± alem or ATG	10 (25.0)
Treo/Cy/flu + alem	1 (2.5)
GVHD prophylaxis, *n* (%)	
CNI + steroids ± MMF ± miraviroc	15 (37.5)
CNI + MMF	12 (30.0)
CNI + MTX ± MMF	5 (12.5)
CNI + MMF + PTCy	1 (2.5)
Sirolimus + MMF	2 (5.0)
CNI	3 (7.5)
MMF	1 (2.5)
None	1 (2.5)

HCT hematopoietic cell transplantation, *MRD* matched related donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *PBSC* peripheral blood stem cell, *flu* fludarabine, *mel* melphalan, *TT* thiotepa, alem alemtuzumab, *ATG* anti-thymocyte globulin, *treo* treosulfan, *Cy* cyclophosphamide, *bu* busulfan, *CNI* calcineurin inhibitor, *MMF* mycophenolate mofetil, *MTX* methotrexate, *PTCy* post-transplant cyclophosphamide

Engraftment and Donor Chimerism

One patient died on day + 23 prior to neutrophil recovery. The remaining patients all achieved primary neutrophil and platelet recovery on median of day + 13 (range 8–60) and + 22.5 (range 9–77), respectively (Table 3). There were no cases of primary graft failure. One patient was classified as having delayed neutrophil engraftment due to receipt of etoposide and other myelotoxic drugs post-HCT, and met the definition of ANC recovery on day +60. Twenty (50%) patients developed mixed chimerism with a median onset of mixed chimerism at 48 days (range 12–917) post-HCT. The lowest whole blood donor chimerism over time ranged from 0 to 92.5% at a median of day + 144 (range 12–1797). Development of mixed donor chimerism was more common in patients who received RIC (18/29 or 62%) compared to patients who received RTC (2/11 or 18%, $p=0.013$). Seven (18%) patients received a cellular intervention for mixed chimerism: two patients received a CD34 + selected stem cell boost plus donor lymphocyte infusion (DLI); one patient received a CD34 + selected stem cell boost without DLI; and 4 patients received DLI alone. Donor chimerism increased to > 95% donor in 4 patients post-CD34 + selected stem cell boost or DLI and in two patients who did not receive additional cellular therapy. Two (5%) patients experienced

Table 3 Transplant outcomes

Characteristic	$N=40$
Engraftment	
Neutrophil (day), median (range)	13 (8–60)
Platelet (day), median (range)	22.5 (9–77)
Mixed chimerism at any time point, *n* (%)	20 (50)
CD34 + stem cell boost and/or DLI, *n* (%)	7 (17.5)
Secondary graft failure, *n* (%)	2 (5)
Second HCT, n (%)	3 (7.5)
HCT toxicities, n (%)	
Grade IV mucositis	1 (2.5)
Veno-occlusive disease	1 (2.5)
Non-infectious pneumonitis	3 (7.5)
Pulmonary hypertension	4 (10)
Clinically significant bleeding	9 (22.5)
Transplant-associated thrombotic microangiopathy	1 (2.5)
Acute GVHD, n (%)	
Grade I	7 (17.5)
Grade II	3 (7.5)
Grade III	4 (10)
Grade IV	2 (5)
Chronic GVHD, n (%), 36 evaluable patients	
Limited	3 (8.3)
Extensive	3 (8.3)
Viral reactivation, n (%)	
EBV	6 (16)
CMV	10 (25)
Adenovirus	7 (17.5)
BK virus	4 (10)
Other	9 (22.5)
Cause of death, n (%)	
Infection	4 (10)
Post-transplant lymphoproliferative disease	1 (2.5)
Acute or chronic GVHD	2 (5)
Non-infectious pneumonitis	2 (5)
Pulmonary hemorrhage	1 (2.5)
Time of death (day), median (range)	280 (23–815)
Follow-up (day), median (range)	751.5 (23–4009)
2-year overall survival (%), (95% CI)	73.6 (55.1–85.5)
2-year event-free survival (%), (95% CI)	63.8 (45.7–77.2)

DLI donor lymphocyte infusion, *HCT* hematopoietic cell transplantation, *GVHD* graft-versus-host disease, *EBV* Epstein-Barr virus, *CMV* cytomegalovirus, *CI* confidence interval

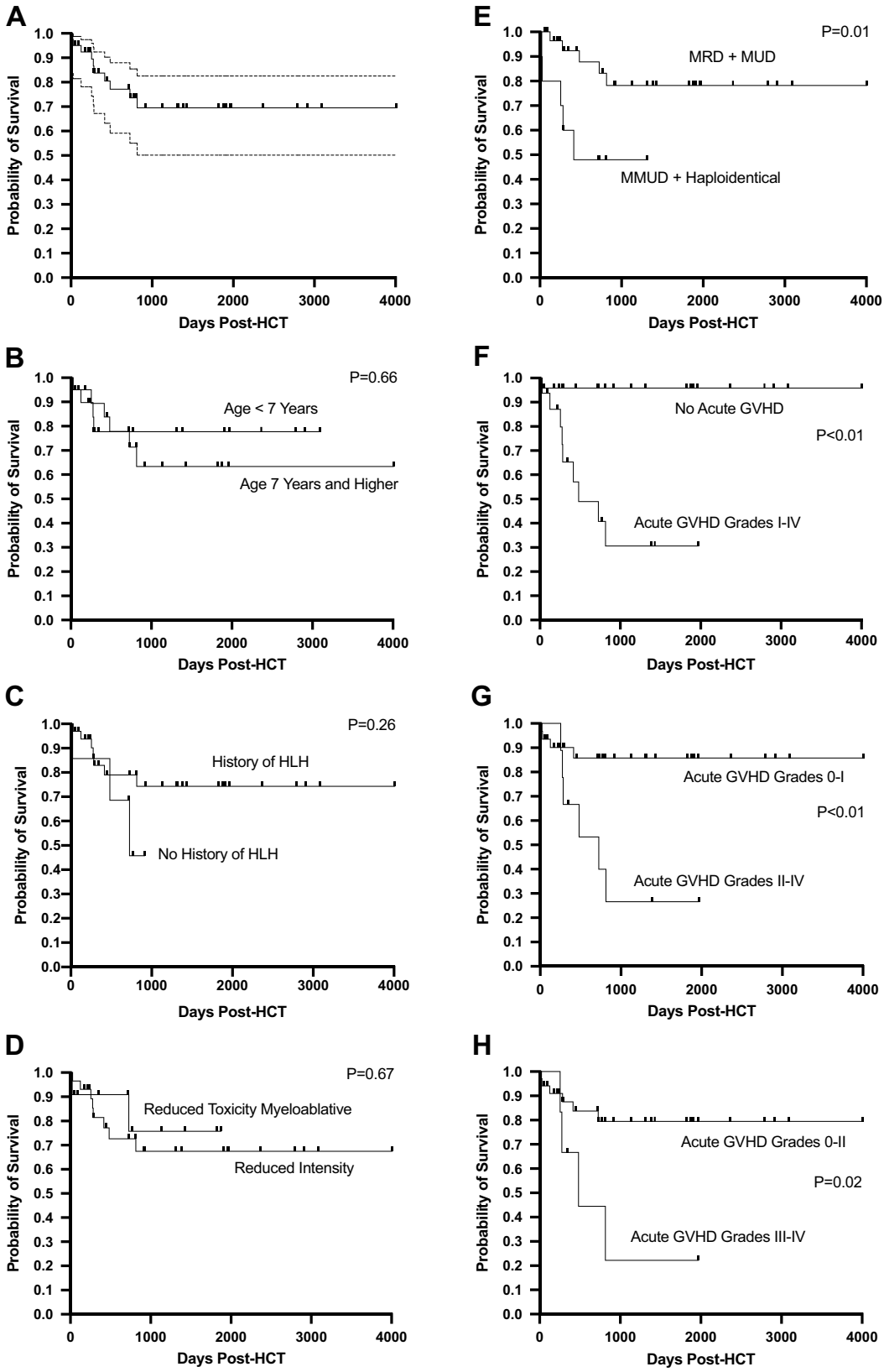
secondary graft failure at 183 and 500 days following HCT. One of the patients experienced graft failure in the setting of HLH relapse and was re-transplanted. The other patient had autologous bone marrow recovery and was clinically stable at last follow-up over 5 years post-HCT. Two (5%) other patients experienced HLH relapse in the setting of mixed donor chimerism (lowest whole blood donor chimerism 11% and 84%), and both went on to receive second transplants. Of note, the patient who received a graft from a female XIAP carrier developed mixed chimerism with whole blood donor chimerism ranging from 10.8 to 52.4% post-transplant. The patient did not experience HLH relapse despite low donor chimerism but developed grade IV acute GVHD following DLI and ultimately died from atypical mycobacterial pneumonia in the setting of extensive chronic GVHD and pulmonary hypertension.

Transplant-Related Toxicities and Morbidities

One (3%) patient developed hepatic veno-occlusive disease from which they successfully recovered. There was 1 (3%) case of transplant-associated thrombotic microangiopathy (TMA). Six (15%) patients developed acute respiratory distress syndrome, and 10 (25%) patients required intubation post-HCT. Non-infectious pulmonary complications were relatively common. Three (8%) patients developed non-infectious pneumonitis, and 4 (10%) patients developed pulmonary hypertension unrelated to infection (one in the setting of pneumonitis). Pulmonary hypertension contributed to death in 2 patients and resolved with time without intervention in the 2 surviving patients. Bleeding was also common post-HCT with life-threatening bleeding reported in 9 (23%) patients; locations of bleeding episodes were the central nervous system ($n=2$), the gastrointestinal tract ($n=5$), and the lungs ($n=2$). Five (13%) patients had renal failure requiring dialysis or continuous renal replacement therapy. Of note, there was no association between non-infectious toxicity and patient age at the time of HCT, conditioning regimen, or presence of HLH before transplantation. Finally, 3 (8%) patients experienced a possible HLH flare in the early post-HCT period, not in the setting of graft failure or mixed chimerism. One patient had an HLH flare/engraftment syndrome on day + 13; one patient developed symptoms concerning for HLH on day + 7 and received a single dose of etoposide on day + 25; and one patient had suspected HLH in the setting of elevated inflammatory markers with severe pneumonitis.

Acute GVHD

Sixteen (40%) patients developed acute GVHD. There were 7 (18%) cases of grade I, 3 (8%) cases of grade II, 4 (10%) cases of grade III and 2 (5%) cases of grade IV acute GVHD. One patient developed acute GVHD in the setting of premature weaning of immune suppression for mixed chimerism, and 3 patients developed acute GVHD following DLI. Notably, both cases of grade IV acute GVHD were following DLI. There was no association between donor source and development of GVHD in this cohort; 6 of 30 (20%) patients who received MRD or MUD grafts developed


A
Probability of Survival
Days Post-HCT
B
P=0.66
Age < 7 Years
Age 7 Years and Higher
C
P=0.26
History of HLH
No History of HLH
D
P=0.67
Reduced Toxicity Myeloablative
Reduced Intensity
E
P=0.01
MRD + MUD
MMUD + Haploidentical
F
No Acute GVHD
P<0.01
Acute GVHD Grades I-IV
G
Acute GVHD Grades 0-I
P<0.01
Acute GVHD Grades II-IV
H
Acute GVHD Grades 0-II
P=0.02
Acute GVHD Grades III-IV

◂**Fig. 1** Overall survival of the **A** cohort as a whole and by **B** age at transplant, **C** history of hemophagocytic lymphohistiocytosis (HLH), **D** conditioning regimen, **E** HLA match (matched related (MRD) and matched unrelated (MUD) versus mismatched unrelated (MMUD) and haploidentical donors), and development of **F** any acute graft-versus-host disease (GVHD), **G** grade II–IV acute GVHD, and **H** grade III–IV acute GVHD

grade II–IV acute GVHD compared to 2 of 10 (20%) patients who received MMUD or haploidentical grafts ($p = 0.80$). Patients who received T cell–replete PBSCs had higher rate of upfront (i.e., before DLI) grade II–IV acute GVHD (4/11 or 36%) than patients who received bone marrow (2/25 or 8%) or T cell–depleted PBSCs (0/4 or 0%), although this difference was not statistically significant ($p = 0.149$). Of note, only one (8%) patient with a history of IBD developed acute gastrointestinal GVHD post-transplant compared to 3 of 27 (11%) patients without a history of IBD (a fourth patient developed grade IV acute gastrointestinal GVHD following DLI).

Chronic GVHD

Thirty-six patients surviving > 100 days after HCT were evaluable for chronic GVHD. Three (8%) patients developed limited skin chronic GVHD, and 3 (8%) patients developed extensive chronic GVHD. The three patients with extensive chronic GVHD all had a history of grade III–IV acute GVHD.

Clinically Significant Viral Reactivation and Other Infections

Twenty-one (53%) patients developed clinically significant viral reactivations: 6 (15%) developed Epstein-Barr virus (EBV) reactivation, 10 (25%) developed cytomegalovirus (CMV) reactivation, 7 (18%) developed adenovirus reactivation, and 4 (10%) developed BK virus hemorrhagic cystitis and/or viremia. Other viral infections included herpes simplex virus type 1 (HSV-1) ($n = 1$, 2.5%), varicella zoster virus (VZV) ($n = 5$, 13%), human herpesvirus 6 (HHV-6) ($n = 3$, 7.5%), and JC virus encephalitis ($n = 1$, 2.5%). Two patients received virus-specific T cells to treat EBV viremia and JC virus encephalitis.

Fourteen (35%) patients had one or more episodes of bacteremia post-HCT. Aspergillus infection was also common post-HCT with confirmed or suspected Aspergillus infection in 6 (15%) patients. Two patients had suspected Aspergillus fungemia based on positive serum Aspergillus antigen; 2 patients had Aspergillus pneumonia; and 2 patients had disseminated aspergillosis. There was 1 (3%) case of Candida fungemia. Of note, the 2 patients with disseminated aspergillosis had grade III acute GVHD or were being treated for HLH relapse (i.e., necessitating immunosuppressive therapy). Other notable infections included atypical mycobacteria multifocal pneumonia in 1 (3%) patient, chronic Norovirus gastroenteritis in 2 (5%) patients and severe warts in 2 (5%) patients.

Survival

Thirty patients were alive at a median of 1025.5 (range 54–4009) days follow-up. The estimated 2-year overall survival was 74% (CI 55–86%) (Fig. 1A), and the estimated 2-year event-free survival was 64% (CI 46–77%). Causes of death included infection ($n = 4$), post-transplant lymphoproliferative disease ($n = 1$), acute GVHD ($n = 1$), chronic GVHD ($n = 1$), pneumonitis ($n = 2$), and pulmonary hemorrhage ($n = 1$). Overall survival was not significantly associated with age at HCT (Fig. 1B, $p = 0.66$) or history of HLH (Fig. 1C, $p = 0.26$) in this cohort. Survival was also similar for patients who received reduced-intensity or reduced-toxicity conditioning regimens (Fig. 1D, $p = 0.67$). Overall survival was, however, significantly lower for patients who received grafts from HLA-mismatched (MMUD or haploidentical) donors compared to patients who received grafts from HLA-matched (MRD or MUD) donors (Fig. 1E, 2-year overall survival of 48% [16–74%] versus 83% [61–93%], $p = 0.01$). Acute GVHD was also strongly associated with poor survival (Fig. 1F–H). The estimated 2-year overall survival was 96% (CI 74–99%) for patients without acute GVHD compared to 41% (CI 15–67%) for patients who developed acute GVHD of any grade ($p < 0.01$). Survival probability decreased with increasing grade of acute GVHD (Fig. 1F–H). Multivariate analysis confirmed recipient and donor HLA match and development of grade II–IV acute GVHD to be associated with a statistically significant adverse impact on survival (Table 4) with hazard ratios of 5.8 (CI 1.5–23.3, $p = 0.01$) and 8.2 (CI 2.1–32.7, $p < 0.01$), respectively. There was no difference in rates of GVHD, including rates of grade III–IV acute GVHD, or other post-transplant complications between patients who received grafts from HLA-matched donors and patients who received grafts from HLA-mismatched donors. The cause of death in patients who received grafts from HLA-mismatched donors was diverse (gram-negative sepsis, post-transplant lymphoproliferative disease, chronic GVHD, pulmonary hemorrhage and pneumonitis).

Resolution of Disease and Quality of Life

As mentioned above, two patients with a history of HLH experienced secondary graft failure, and two other patients experienced HLH relapse in the setting of mixed donor chimerism (lowest whole blood donor chimerism 11% and

Table 4 Multivariate analysis of risk of death

Parameter	*N* (*N* event)	HR (95% CI)	*p* value
HLA match and relation			
MRD or MUD	30 (5)	1.0	
MMUD or haplo	10 (5)	5.8 (1.5–23.3)	0.01
Acute GVHD			
Grades 0–I	31 (4)	1.0	
Grades II–IV	9 (6)	8.2 (2.1–32.7)	<0.01

Final model shown. Variables considered are as follows: age at HCT, history of HLH, recipient, and donor HLA match and relation, conditioning regimen intensity, and grade II–IV acute GVHD

84%). The remaining 21 surviving patients with a history of HLH or recurrent fevers with or without cytopenia pre-HCT have not experienced disease relapse with whole blood donor chimerism ranging from 50 to 100% at last follow-up. Twelve of 13 (92%) patients with IBD pre-HCT had resolution of IBD post-HCT with whole blood donor chimerism ranging from 74 to 100% at last follow-up. The thirteenth patient had chronic norovirus and sapovirus gastroenteritis and gastrointestinal GVHD, making it difficult to determine resolution of underlying IBD. Twenty-two of 26 (85%) surviving patients who did not proceed to the second HCT were off *Pneumocystis jirovecii* prophylaxis at last assessment, and twenty (77%) patients were off immunoglobulin replacement. Median Lansky or Karnofsky score was 100 (range 70–100) at last follow-up at a median of 1131 days (range 54–3088) post-transplant.

Discussion

Here, we report the outcomes of allogeneic HCT in the largest cohort of patients with XIAP deficiency transplanted to date. Our data suggest that XIAP-deficient patients tolerate reduced-intensity and reduced-toxicity conditioning approaches. Rates of serious infectious and non-infectious post-HCT complications were high, with 35% of patients experiencing life-threatening bleeding or requiring ventilation with or without dialysis or continuous renal replacement therapy. However, despite the high rate of transplant-associated toxicity, the 2-year overall survival in our cohort was 74%, which is very similar to the 71% 3-year overall survival reported by the CIBMTR for 413 patients with genetic HLH diseases transplanted between 2010 and 2016. [35] Our data are reassuring to physicians considering allogeneic HCT for XIAP-deficient patients with severe or refractory disease phenotypes, though obviously, there still remains a significant risk of morbidity and mortality with allogeneic HCT.

Notably, survival in our series was exceptionally impacted by the occurrence of acute GVHD. Murine models of HCT have previously demonstrated an adverse impact of recipient XIAP deficiency on GVHD severity and mortality, [30, 31] and the pan-inhibitor of apoptosis inhibitor AT-406 has also been shown to aggravate experimental GVHD. [30] Experiments that examined the critical cell types for exacerbation of GVHD found that only recipient XIAP deficiency was important; murine donor T cells that were XIAP-deficient had no impact. [30, 31] Beyond this, some results have been inconsistent. Muller et al. observed increased tissue infiltration of T cells and increased levels of interferon-gamma in XIAP-deficient recipients and concluded that this was secondary to enhanced stimulation by antigen-presenting cells. T cells produced higher levels of interferon-gamma in in vitro mixed lymphocyte reactions when co-cultured with XIAP-deficient bone marrow–derived dendritic cells which had increased levels of IL-1beta secretion. [31] Muller et al. also observed that chimeric recipient mice with XIAP-deficient hematopoietic systems experienced worse GVHD. [31] However, Toubai et al. did not replicate these observations. [30] They found that recipient non-hematopoietic cell XIAP deficiency was critical for exacerbating GVHD, and increased mortality. [30] They also reported increased intestinal epithelial cell apoptosis in XIAP-deficient recipient mice. [30] In summary, murine models suggest mechanisms whereby both residual recipient XIAP-deficient tissue antigen-presenting cells and non-hematopoietic cells cause increased GVHD severity following allograft HCT. The relative importance of these two cell types, however, remains uncertain.

Regardless, these studies make it clear that human patients, who are XIAP-deficient in both hematopoietic-derived and non-hematopoietic-derived cells, may be at risk of increased GVHD severity and mortality. Indeed, our findings demonstrate a significant negative impact of acute GHVD on survival in patients with XIAP deficiency. It is possible that patients experience exaggerated local tissue inflammation in the setting of allogeneic responses due to the XIAP-deficient tissue environment with increased epithelial and/or antigen-presenting cell cytokine production and cell death early after transplant. Patients with XIAP deficiency are known to have high baseline levels of IL-18, [16] indicating chronic inflammasome activity, which might also contribute to local tissue pathology early after transplant. Thoughtful consideration should be given to the aggressive prevention of GVHD in patients with XIAP deficiency, for example, through the use of ex vivo T cell–depleted stem cell grafts for patients without a suitable HLA-matched donor available. Careful consideration should also be given when confronted with mixed recipient and donor chimerism. Three patients in this cohort developed acute GVHD following DLI for mixed chimerism, and notably, both cases of grade IV

acute GVHD were following DLI from a matched sibling donor. CD34 + selected stem cell boosts may be a better option for these patients, but ultimately, further fine tuning of conditioning regimens to decrease the risk of mixed chimerism without concomitantly increasing toxicity is needed. Reassuringly, survival in our cohort without acute GVHD was 96%. Also reassuring is that we and others have observed resolution of IBD, indicating no evidence of persistent clinically significant propensity to pathologic tissue inflammation following allogeneic HCT.

One limitation of this study includes its retrospective nature with a variety of donor types, graft sources, and conditioning regimens employed, limiting our ability to provide recommendations for optimal graft sources and conditioning regimens for patients with XIAP deficiency. Selection bias may also exist. However, the data as such suggests that both RIC and RTC regimens are tolerated with similar transplant outcomes. In addition, only whole blood donor chimerism was collected for most patients at various time points post-transplant. It would be helpful to have uniform myeloid and lymphoid subset chimerism studies for all patients to analyze the minimum level of donor chimerism needed to prevent disease relapse (both HLH and IBD). Nevertheless, this study further supports the resolution of both HLH and IBD phenotypes following successfully sustained engraftment in patients with XIAP deficiency.

In summary, we have demonstrated that survival rates for patients with XIAP who are transplanted with reduced-intensity and reduced-toxicity approaches are similar to other patients with genetic HLH disorders. Measures should be taken to prevent acute GVHD as much as possible in XIAP-deficient patients who undergo allogeneic HCT.

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Author Contribution DEA analyzed the data and wrote the manuscript. RAM and AW contributed patient data, analyzed data, and edited the manuscript. RN and CW analyzed the data and edited the manuscript. KL, KW, MHA, ECM, JRH, NJB, AK, MBJ, TC, SC, TB, CS, SE, MS, JW, KR, and CB contributed patient data and edited the manuscript.

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval The study was approved by the Cincinnati Children's Hospital institutional review board.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest JW is employed by AbbVie. AK and MBJ have done ad hoc consultancy for Swedish Orphan Biovitrum. CB has done ad hoc consultancy for Novimmune and Swedish Orphan Biovitrum. The remaining authors have no relevant conflicts of interest to disclose.

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Authors and Affiliations

Danielle E. Arnold[1] · Rofida Nofal[2] · Connor Wakefield[3] · Kai Lehmberg[4] · Katharina Wustrau[4] · Michael H. Albert[5] · Emma C. Morris[6] · Jennifer R. Heimall[7] · Nancy J. Bunin[8] · Ashish Kumar[1] · Michael B. Jordan[1] · Theresa Cole[9] · Sharon Choo[9] · Tim Brettig[9] · Carsten Speckmann[10] · Stephan Ehl[10] · Malgorzata Salamonowicz[11] · Justin Wahlstrom[12] · Kanchan Rao[13] · Claire Booth[14] · Austen Worth[14] · Rebecca A. Marsh[1]

1 Division of Bone Marrow Transplantation and Immune Deficiency, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, Cincinnati, OH 45229, USA

2 CA, San Francisco, USA

3 Rush Medical College, Rush University Medical Center, Chicago, IL, USA

4 Division of Pediatric Stem Cell Transplantation and Immunology, University Medical Center Hamburg, Hamburg, Germany

5 Dr. Von Hauner Children's Hospital, University Hospital, Ludwig-Maximilians University, Munich, Germany

6 Institute of Immunity and Transplantation, University College London, London, UK

7 Division of Allergy and Immunology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

8 Division of Oncology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

9 Department of Allergy and Immunology, The Royal Children's Hospital, Melbourne, VIC, Australia

10 Institute for Immunodeficiency, Center for Chronic Immunodeficiency, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany

11 Department of Pediatric Stem Cell Transplantation, Hematology and Oncology, Medical University, Wroclaw, Poland

12 Blood and Marrow Transplantation Program, Benioff Children's Hospital, University of California San Francisco, San Francisco, CA, USA

13 Department of Bone Marrow Transplantation, Great Ormond Street Hospital for Children, London, UK

14 Department of Pediatric Immunology, Great Ormond Street Hospital, London, UK