REGULAR ARTICLE

blood advances

Real-world experience with low-dose IL-2 for children and young adults with refractory chronic graft-versus-host disease

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Key Points

- Low-dose IL-2 is safe and well tolerated at variable dosing regimens in pediatric patients with steroid-refractory chronic GVHD.
- Low-dose IL-2 is effective in combination with other immunosuppressive medications.

The majority of patients with chronic graft-versus-host disease (cGVHD) are steroid refractory (SR), creating a need for safe and effective therapies. Subcutaneous low-dose interleukin-2 (LD IL-2), which preferentially expands $CD4^+$ regulatory T cells (Tregs), has been evaluated in 5 clinical trials at our center with partial responses (PR) in ~50% of adults and 82% of children by week 8. We now report additional real-world experience with LD IL-2 in 15 children and young adults. We conducted a retrospective chart review of patients with SR-cGVHD at our center who received LD IL-2 from August 2016 to July 2022 not on a research trial. The median age at start of LD IL-2 was 10.4 years (range, 1.2-23.2 years) at a median of 234 days from cGVHD diagnosis (range, 11-542 days). Patients had a median of 2.5 (range, 1-3) active organs at LD IL-2 start and received a median of 3 (range, 1-5) prior therapies. The median duration of LD IL-2 therapy was 462 days (range, 8-1489 days). Most patients received 1×10^6 IU/m^2 per day. There were no serious adverse effects. The overall response rate in 13 patients who received >4 weeks of therapy was 85% (complete response, n = 5; PR, n = 6) with responses in diverse organs. Most patients significantly weaned corticosteroids. Tregs preferentially expanded with a median peak fold increase of 2.8 in the ratio of Tregs to $CD4^+$ conventional T cells (range, 2.0-19.8) by 8 weeks on therapy. LD IL-2 is a well-tolerated, steroid-sparing agent with a high response rate in children and young adults with SR-cGVHD.

Introduction

Chronic graft-versus-host disease (cGVHD) is a multisystem disorder that complicates allogeneic hematopoietic cell transplantation (HCT) in between 20% and 50% of children.[1] It arises from a failure of donor-derived B and T cells to develop tolerance to allo- and autoantigens during immune reconstitution after transplantation.[1,2] The immune-mediated damage in cGVHD eventually results in fibrotic changes that can affect any organ system and can lead to debilitating complications such as sclerotic skin, joint contractures, and bronchiolitis obliterans syndrome. Given these serious multiorgan effects, cGVHD is the leading cause of nonrelapse morbidity and mortality after allogeneic HCT.[1,2]

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The full-text version of this article contains a data supplement.

The development of cGVHD is driven by tissue damage that primes dendritic cells to display costimulatory molecules to T cells.[3] Subsequently, allo- and autoreactive T cells can activate pathogenic B cells, which release damaging immunoglobulins. Lymphocyte activation is accompanied by macrophage activation and release of profibrotic growth factors.[3] Because of thymic injury from transplant conditioning and T-cell alloreactivity, central tolerance is impaired. Poor reconstitution of the $CD4^+$ regulatory T-cell (Treg) compartment after transplantation also leads to dysfunctional peripheral tolerance.[3-7] The failures in central and peripheral tolerance enable unchecked inflammation to drive fibrosis.

With broad immunosuppressive effects, corticosteroids remain the first-line therapy for cGVHD[8]; however, prolonged steroid exposure is associated with significant morbidity, and between 40% and 50% of patients are steroid refractory (SR).[9] There are now 3 US Food and Drug Administration (FDA)-approved second-line agents for treatment of refractory cGVHD: ibrutinib, ruxolitinib, and belumosudil, with overall response rates (ORR) of 50% to 77% in phase 2 and 3 adult studies.[10,11] Ibrutinib inhibits Bruton tyrosine kinase and IL-2 inducible T-cell kinase, targeting pathogenic B- and T-cell activation, respectively. Ruxolitinib is a Janus kinase (JAK) inhibitor that blocks signaling through multiple cytokine receptors on lymphocytes. Finally, belumosudil inhibits Rho-associated coiled coil–containing protein kinase-2, which prevents STAT3 phosphorylation and development of Th17 and T-follicular helper cell populations.[3]

An alternative approach to refractory cGVHD developed at our center over the last 15 years is to augment the Treg compartment using low-dose interleukin-2 (LD IL-2). IL-2 is an essential cytokine for the proliferation and function of both regulatory and effector T cells. However, only Tregs constitutively express the higher affinity trimeric $\alpha\beta\gamma$ IL-2 receptor, enabling the preferential expansion of Tregs with low doses of IL-2.[12] There have been 5 clinical trials investigating daily subcutaneous injections of LD IL-2 for SR-cGVHD at the Dana-Farber Cancer Institute (NCT00529035, NCT01366092, NCT01937468, NCT02318082, and NCT02340676), one of which included pediatric patients.[13-18] In this phase 1 intrapatient dose-escalation study (NCT02318082), pediatric patients tolerated the previously defined maximum tolerated dose in adults of 1×10^6 IU/m^2 per day. By 8 weeks, 82% (9 of 11) of children attained a partial response (PR) and 2 patients attained a complete response (CR) on extended-duration therapy. This ORR was higher than the 50% ORR typically observed among adult participants in our LD IL-2 trials. As a result, LD IL-2 is now offered as an off-trial second-line option for pediatric patients with SR-cGVHD at our center.

Data from clinical trials are often complemented by observations from real-world clinical use, which is less restrictive and more generalizable to diverse settings. To date, to the best of our knowledge, there are no reports of such data for LD IL-2 in pediatric patients for any disease indication. Here, we present our experience using LD IL-2 off study in pediatric and young adult patients with SR-cGVHD.

Methods

Patient selection

We conducted a retrospective chart review of all patients who received LD IL-2 for SR-cGVHD at the Dana-Farber/Boston Children's Cancer and Blood Disorders Center while not on a clinical trial between August 2016 and July 2022. We collected patient demographic and transplant-related data via a data pull from Ottr (CareDx), an institutional data repository for patients who have received HCT. Race and ethnicity data recorded in this database are per patient self-report. We collected data for cGVHD involvement and severity, medication exposures, infectious or other complications, and disease response by manual chart review. Patients who received <4 weeks of therapy were considered inevaluable for clinical response based on experience from our prior studies in which clinical benefit required at least 8 to 12 weeks of therapy.[13-18] This study was approved by the Dana-Farber/Harvard Cancer Center institutional review board (DFCI 21-417) and conducted in accordance with the Declaration of Helsinki.

LD IL-2 preparation

Recombinant IL-2 (aldesleukin; Clinigen) was provided as a lyophilized powder in vials of 22 million IU. Vials were aseptically diluted at the Dana-Farber Cancer Institute pharmacy by qualified personnel in a laminar flow hood with 1.2 mL sterile water and 4.8 mL dextrose 5% in water, resulting in a final IL-2 concentration of 3.67 million IU/mL. After reconstitution, a 14-day supply was provided to families in single-use syringes for home refrigerator storage at 2 to 8°C.

Clinical assessments

We determined clinical response and potential adverse events related to LD IL-2 by reviewing clinic visit and stem cell transplant inpatient notes in the electronic medical record, starting the month before LD IL-2 initiation through the end date of LD IL-2 therapy. Two to 3 physicians (H.W., J.S.W., and M.K.) adjudicated the response for each patient, and responses were based on 2014 National Institutes of Health (NIH) consensus criteria.[19,20]

Immune cell analysis

Cell counts and lymphocyte populations data were collected as available in the medical record. Flow cytometry subsets performed through the Boston Children's Hospital clinical laboratory included $CD19^+$ cells (B cells), $CD3^+$ cells (T cells) with $CD4^+$ and $CD8^+$ subsets, as well as $CD4^+$ $CD25^+$ $CD127^{low}$ cells ($CD4^+$ Tregs). Natural killer (NK) cells were identified as $CD3^-$ and either $CD16^+$ or $CD56^+$.

Statistical analysis

Data analysis was primarily descriptive. The Wilcoxon signed-rank test was used for paired comparison, and multiple comparisons were not considered. Statistical analyses were conducted using GraphPad Prism version 9 (San Diego) and R version 4.2.2 (the CRAN project).

Results

Patient characteristics

Fifteen patients received LD IL-2 for SR-cGVHD off study at the Dana-Farber/Boston Children's Cancer and Blood Disorders Center between August 2016 and July 2022. This cohort included 2 patients (patient [P]7 and P12) who restarted, or continued, LD IL-2 therapy off study after initially being treated on the phase 1 trial (NCT02318082). Baseline patient characteristics are shown in

Table 1. Patient baseline characteristics

	Number (N = 15)	Percentage (%)
Age at transplant (y), median (range)	9.1 (0.3-21.4)	
Sex		
Male	11	73%
Female	4	27%
Race		
White	8	53%
Black	2	13%
Asian/Pacific Islander	1	7%
Other	3	20%
Not disclosed	1	7%
Ethnicity		
Non-Hispanic/Latino	8	53%
Hispanic/Latino	4	27%
Not disclosed	3	20%
Diagnosis		
Nonmalignant	8	53%
Malignant	7	47%
Conditioning		
MAC	10	67%
RIC	3	20%
None	1	7%
Unknown*	1	7%
HLA type (A, B, C, DRB1)		
Matched unrelated	5	33%
Matched related	3	20%
Mismatched unrelated	6	40%
Haploidentical	1	7%
Cell source		
Bone marrow	13	87%
Peripheral blood	1	7%
Unknown*	1	7%
Prior aGVHD		
Yes	9	60%
No	5	33%
Unknown*	1	7%
Time to cGVHD from HCT (d), median (range)	168 (100-420)	
Time to LD IL-2 from cGVHD diagnosis (d), median (range)	234 (11-542)	
Age at LD IL-2 start (y), median (range)	10.4 (1.2-23.2)	
Time on LD IL-2 (d), median (range)	462 (8-1489)	

aGVHD, acute GVHD; MAC, myeloablative conditioning; RIC, reduced intensity conditioning.
*One patient had received transplantation elsewhere with few records before her presentation for evaluation of her cGVHD.

Table 1. Seventy-three percent (11 of 15) of patients were male; 53% (8 of 15) of patients self-identified as White. The median age at stem cell transplantation was 9.1 years (range, 0.3-21.4 years). Fifty-three percent (8 of 15) of patients underwent transplantation for nonmalignant indications, 67% (10 of 15) received myeloablative conditioning, 40% (6 of 15) had mismatched unrelated donors, and 87% (13 of 15) received bone marrow as the stem cell source. Baseline transplant details were not available for 1 patient (P15) who received her stem cell transplant in another country.

cGVHD characteristics

The median time from HCT to cGVHD diagnosis was 168 days (range, 100-420 days), and the median time from cGVHD to LD IL-2 initiation was 234 days (range, 11-542 days). The median age at the start of LD IL-2 treatment was 10.4 years (range, 1.2-23.2 years). Patients tried a median of 3 (range, 1-5) therapies (including steroids) before IL-2 and had a median of 2.5 (range, 1-3) actively affected organs at the time of IL-2 treatment initiation. All patients had moderate-to-severe cGVHD per 2014 NIH consensus criteria.[19] Individual patient characteristics including transplant indication, comorbidities, cGVHD features, and response to LD IL-2 are provided in Table 2.

LD IL-2 safety and tolerability

The median duration on therapy for our cohort was 462 days (range, 8-1489 days). Most patients received 1×10^6 IU/m^2 per day, which was the previously identified maximum tolerated dose.[20] However, 3 patients (P2, P9, and P15) requested a lowering of the dose because of discomfort or malaise associated with the daily subcutaneous injections (decreased to 0.33×10^6 IU/m^2 per day for P2; 1×10^6 IU/m^2 every other day for P9 and P15), which subsequently improved. P8 had dose reduction to 50% in the setting of reduced renal function. Most dose reductions occurred within the first 2 months of therapy.

No patient with a transplant indication of malignancy experienced relapse on LD IL-2 nor were there any serious adverse events directly attributable to LD IL-2 therapy. However, there were several adverse events that occurred during IL-2 therapy. P4 had a flare of preexisting alloimmune-mediated chronic relapsing encephalomyelitis upon steroid wean. P12 had a recurrence of pancreatitis after restarting steroids, and P15 had a recurrence of a multidrug resistant *Pseudomonas* breast abscess. In addition, 2 patients developed new infections, which occurred in the setting of multiple immune suppressive agents and cannot be attributed to LD IL-2 alone. P2 and P3 developed pulmonary aspergillosis during LD IL-2 therapy while also receiving 10 mg twice daily of ruxolitinib.

Clinical response and survival outcomes

Thirteen of 15 patients were evaluable for clinical response based on duration of therapy of >4 weeks. Two patients (P8 and P11) received <4 weeks of therapy (27 and 8 days, respectively) and were not considered evaluable for response. The ORR was 85% (11 of 13), comprised of 5 CR and 6 PR. The median time required to achieve PR in any involved organ was 8.3 weeks (range, 4-69 weeks) (Figure 1). There were 4 organ-specific PR by 6 weeks (2, skin; 1, liver; 1, joints-muscle-fascia). The median time to CR in any involved organ was 27 weeks (range, 8-80 weeks). Two patients (P7 and P10) had stable disease (SD). P10 had improvement of alopecia and vitiligo from cGVHD but these features are not included in the NIH skin scoring system.

Patients P7 and P12 were initially enrolled in the pediatric cohort of the phase 1 clinical trial (NCT02318082) of intrapatient dose-escalated LD IL-2.[18] P7, with isolated lung involvement, had a PR

Table 2. Details on individual patients and IL-2 course

	Transplant indication	cGVHD features	Previous GVHD therapies*	Other previous comorbidities	Age at LD IL-2 start (y)	Clinical response (excluding oral/ocular)	Adverse events during therapy (not thought to be from LD IL-2)	Reason for IL-2 dose change or discontinuation
P1	Fanconi anemia	Skin Ocular	Steroid Sirolimus	• Acute GVHD: gut	10.6	Overall CR Skin CR		cGVHD stable/improved, thus weaned off therapy
P2	AML	Lung JMF	Steroid Imatinib Ruxolitinib		18.1	Overall PR Lung SD JMF PR	Pulmonary aspergillosis	Reduced to 0.33 dosing because of pain related to injections Stopped because of pulmonary aspergillosis
P3	Mixed leukemia	Liver Ocular Lung†	Steroid Ruxolitinib Vedolizumab	• Acute GVHD: skin, oral, gut, liver • Stem cell boost for low CD34 counts • Eosinophilic gastroenteritis	18.4	Overall PR Liver CR Lung SD	Pulmonary aspergillosis with development of pulmonary GVHD	6.5 mos break after aspergillosis; then restarted LD IL-2; cGVHD stable/improved, thus weaned off therapy
P4	Immune deficiency (WAS)	Lung	Steroid	• Initial graft failure followed by second transplant • Chronic relapsing encephalomyelitis	4.3	Overall PR Lung PR		cGVHD stable/improved, thus weaned off therapy
P5	fHLH (STXBP2)	Skin Gut Ocular	Steroid Tacrolimus Vedolizumab* Ruxolitinib*	• Acute GVHD: skin, gut • Recurrent CVL infection • Waning chimerism → stem cell boost	4.6	Overall CR Skin CR Gut (not active at time of start)		cGVHD stable/improved, thus weaned off therapy
P6	Omenn syndrome (IL-7R deficiency)	Skin Gut	Steroid	• Acute GVHD: gut • Feeding intolerance; on parenteral nutrition • Central sleep apnea • Diabetes • CMV and EBV viremia • Tracheostomy with recurrent tracheitis and bacteremia	1.2	Overall PR Skin CR Gut PR	Idiopathic giant cell myocarditis	Held during infections & cardiac workup (2 mo) IL-2 eventually paused for trial of mepolizumab (unclear if there would be an interaction)
P7	Immune deficiency (unknown)	Lung	Steroid Cyclosporine MMF	• Central nervous system EBV^+ leiomyosarcoma (resected ×3) • Chronic CMV viremia • Toxic epidermal necrolysis	8.4	Overall SD Lung SD	Influenza with multifocal bacterial pneumonia	Was initially enrolled on pediatric cohort of LD IL-2 trial and had PR in lungs at week 8. Came off trial at week 97 because of progression in setting of respiratory infection Restarted IL-2 off study 2 mos later. cGVHD stable/improved, thus weaned off therapy
P8	AML	Liver Ocular Oral	Steroid Ibrutinib* Infliximab*	• Second transplant after initial relapse of AML • Severe malnutrition • Organizing pneumonia	18.4	Inevaluable		50% dosing for prerenal AKI Severe liver failure at time of IL-2 start; goals of care soon redirected to comfort measures
P9	ALD	Liver Ocular JMF	Steroid Cyclosporine Rituximab* Ibrutinib* Ruxolitinib*		10.2	Overall CR Liver CR JMF CR		Fatigue & flushing; switched to every-other-day dosing; cGVHD stable/improved, thus weaned off therapy
P10	AML	Lung Skin‡	Steroid Cyclosporine Ruxolitinib	• Acute GVHD: skin • Pulmonary superinfection & scarring • HHV-6 & BK Virus	12.7	Overall SD Lung SD	*Pseudomonas* pneumonia	Insurance stopped covering

AKI, acute kidney injury; ALD, adrenoleukodystrophy; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; BK, BK virus; CMV, cytomegalovirus; CVL, central venous line; EBV, Epstein-Barr virus; ERCP, endoscopic retrograde cholangiopancreatography; fHLH, familial hemophagocytic lymphohistiocytosis; HHV-6, human herpesvirus 6; ITK, interleukin-2-inducible T-cell kinase; JMF, joints, muscles, fascia; MDR, multidrug resistant; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; PsA, *Pseudomonas*; WAS, Wiskott-Aldrich syndrome.

*Indicates had been stopped before starting IL-2; otherwise, therapies active at time of IL-2 start.

†Developed lung involvement after start of IL-2 in the setting of aspergillosis.

‡Hypopigmentation, vitiligo; not part of official NIH scoring therefore not used for response (although improved).

Table 2 (continued)

Transplant indication	cGVHD features	Previous GVHD therapies*	Other previous comorbidities	Age at LD IL-2 start (y)	Clinical response (excluding oral/ocular)	Adverse events during therapy (not thought to be from LD IL-2)	Reason for IL-2 dose change or discontinuation
P11 SAA	Skin Liver Lung	Steroid Cyclosporine Ruxolitinib	• Acute GVHD: liver, gut • Thrombocytopenia	10.2	Inevaluable		Stopped IL-2 & ruxolitinib because of thrombocytopenia; later the cause was determined to be immune thrombocytopenia
P12 Gamma-delta T-cell lymphoma	Skin Liver Lung	Steroid	• Acute GVHD: liver, gut, skin • Polymicrobial infected hemorrhagic pancreatic pseudocyst → distal pancreatectomy, splenectomy, & near-total colectomy • Steroid-induced chronic kidney disease	23.2	Overall CR Skin CR Liver CR Lung CR	Pancreatitis	On study for 122 weeks and then continued off-study for 90 weeks cGVHD stable/improved, thus weaned off therapy
P13 Immune deficiency (ITK deficiency)	Skin Gut	Steroid Tacrolimus Vedolizumab	• Acute GVHD: skin • Liver transplant before HCT for genetic liver failure; complicated by wound dehiscence and intra-abdominal infection requiring abdominal wash out and biliary duct dilation requiring ERCP and stent placement in the common bile duct. • Poor graft function despite stem cell boost • Persistent HHV-6 viremia • Chronic pericardial effusion	4.1	Overall CR Skin CR Gut CR	Bacteremia	Given persistent graft failure, infections, and multiorgan dysfunction, switched to comfort care
P14 B-ALL	Skin Liver Oral	Steroid MMF	• Acute GVHD: skin	10.4	Overall PR Skin SD Liver PR		Mild improvement in skin but early plateau; added ruxolitinib & stopped IL-2
P15 Myeloproliferative disorder (JAK2 mutation)	Skin Liver Ocular	Steroid Ruxolitinib	• Acute GVHD status unknown • Bilateral breast tissue ulceration/ sclerodermatous change with MDR PsA that preceded starting IL-2	15.9	Overall PR Skin PR Liver CR	Recurrence of breast tissue infection	Malaise at daily dosing, switched to every other day Discontinued, because unavailable in home country

AKI, acute kidney injury; ALD, adrenoleukodystrophy; AML, acute myeloid leukemia; B-ALL, B-cell acute lymphoblastic leukemia; BK, BK virus; CMV, cytomegalovirus; CVL, central venous line; EBV, Epstein-Barr virus; ERCP, endoscopic retrograde cholangiopancreatography; fHLH, familial hemophagocytic lymphohistiocytosis; HHV-6, human herpesvirus 6; ITK, interleukin-2-inducible T-cell kinase; JMF, joints, muscles, fascia; MDR, multidrug resistant; MMF, mycophenolate mofetil; SAA, severe aplastic anemia; PsA, *Pseudomonas*; WAS, Wiskott-Aldrich syndrome.

*Indicates had been stopped before starting IL-2; otherwise, therapies active at time of IL-2 start.

†Developed lung involvement after start of IL-2 in the setting of aspergillosis.

‡Hypopigmentation, vitiligo; not part of official NIH scoring therefore not used for response (although improved).

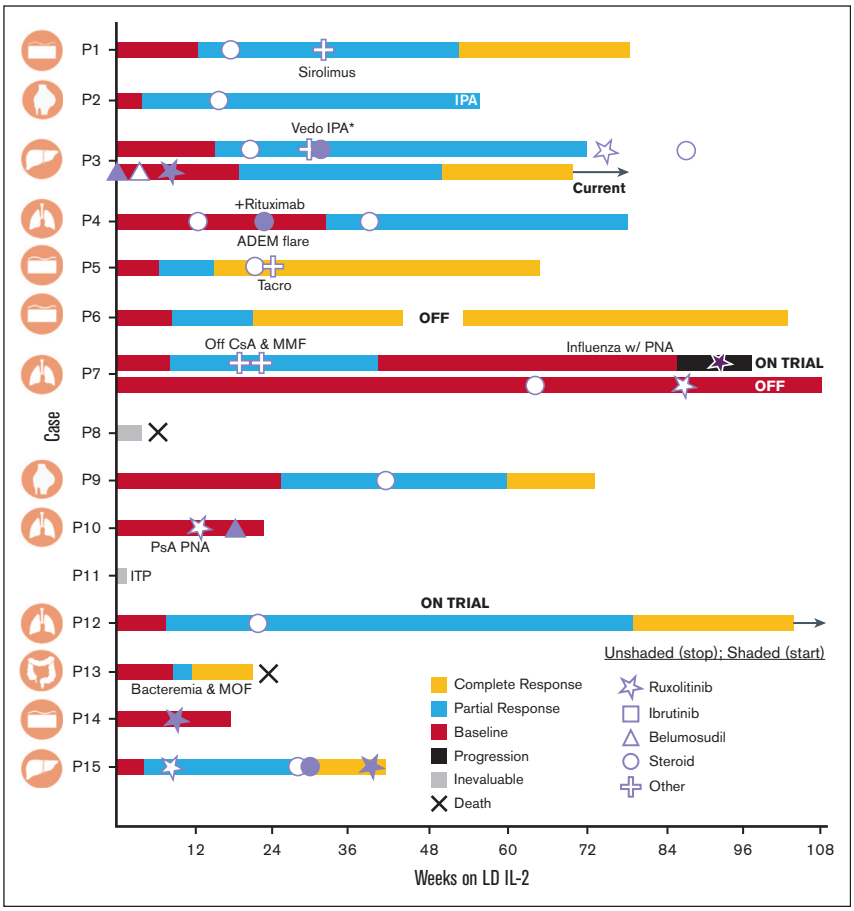

Figure 1. Swimmer plot outlining the timeline for clinical response in each patient's best-response organ. These courses may be cross referenced against details in Table 2, which shows cotherapies and active organs at the time of LD IL-2 start (time 0 on x-axis). CsA, cyclosporine; IPA, invasive pulmonary aspergillosis; ITP, immune thrombocytopenia; MMF, mycophenolate mofetil; MOF, multiorgan failure; PsA, *Pseudomonas*; PNA, pneumonia; Tacro, tacrolimus; Vedo, vedolizumab.

in the lungs at week 8 on study and continued extended-duration therapy for another 89 weeks before discontinuing because of progressive disease in the setting of a pulmonary infection. However, his lung cGVHD progressed even more rapidly when IL-2 was briefly discontinued. Thus, he was restarted on LD IL-2 off study, 9 weeks later, and was able to maintain SD over the next 2 years. P12 received 122 weeks of therapy on the same phase 1 clinical trial but had to discontinue on study because of shortage of study drug supply. However, he was able to obtain insurance coverage for aldesleukin and continued receiving LD IL-2 off study for another 90 weeks without interruption. The follow-up time in the prior publication was not long enough to capture his CR for lung at 80 weeks of therapy, and we include his full course to demonstrate safety for a total of 212 weeks of therapy.[18]

Several patients with multiorgan involvement with an overall PR had organ-specific CR. Figure 2A-B show the organ-specific response rate in our cohort. The highest rates of CR were seen for liver (4 of 5 patients) and skin GVHD (5 of 7 patients), the latter of which reflects improvements in both erythroderma/desquamation as well as scleroderma (Figure 2C and D, respectively). In the 6 patients with lung involvement, there was 1 CR, 1 PR, and 4 SD based on formal NIH response criteria. However, several of the patients with SD showed improvement of percentage predicted forced expiratory volume in the first second (%FEV1) by pulmonary function testing despite not meeting objective PR criteria (supplemental Figure 1). For example, P2 initially had improvement in %FEV1 from 46% at baseline to 54% at 36 weeks on therapy, although she subsequently had worsening back down to 43%, 14 weeks later, after a presumed pulmonary aspergillosis infection. P3 developed new-onset bronchiolitis obliterans during his first course of LD IL-2 after pulmonary aspergillosis but then had improvement in %FEV1 from 30% at the start of his second course of LD IL-2 to 37% after 37 weeks of therapy. The CR shown for lung in P12 reflects pulmonary GVHD considered to be mixed cryptogenic organizing pneumonia/bronchiolitis obliterans syndrome. Of note, P12 also achieved CR of his liver and skin cGVHD.

In addition to improvement in clinical features, many patients were able to significantly wean corticosteroid dose while receiving LD IL-2 (Figure 3A-B). All of the patients who were evaluable (n = 13) were on corticosteroids at LD IL-2 start with a median steroid dose of 0.79 mg/kg per day (range, 0.13-1 mg/kg per day). Most providers chose to wait until at least 4 weeks on LD IL-2 therapy before attempting a steroid wean. By week 8, the median dose was 0.50 mg/kg per day (range, 0.07-1 mg/kg per day) and median reduction from baseline was 30% (range, −72% to 0%) (P = 0.002). The median dose at IL-2 discontinuation was 0.09 mg/kg per day (range, 0-0.94 mg/kg per day) and median reduction from baseline was 56% (range, −100% to 30.8%) (P = 0.003) (supplemental Table 1). Six patients were able to permanently discontinue corticosteroid therapy after 16 to 20 weeks on LD IL-2.

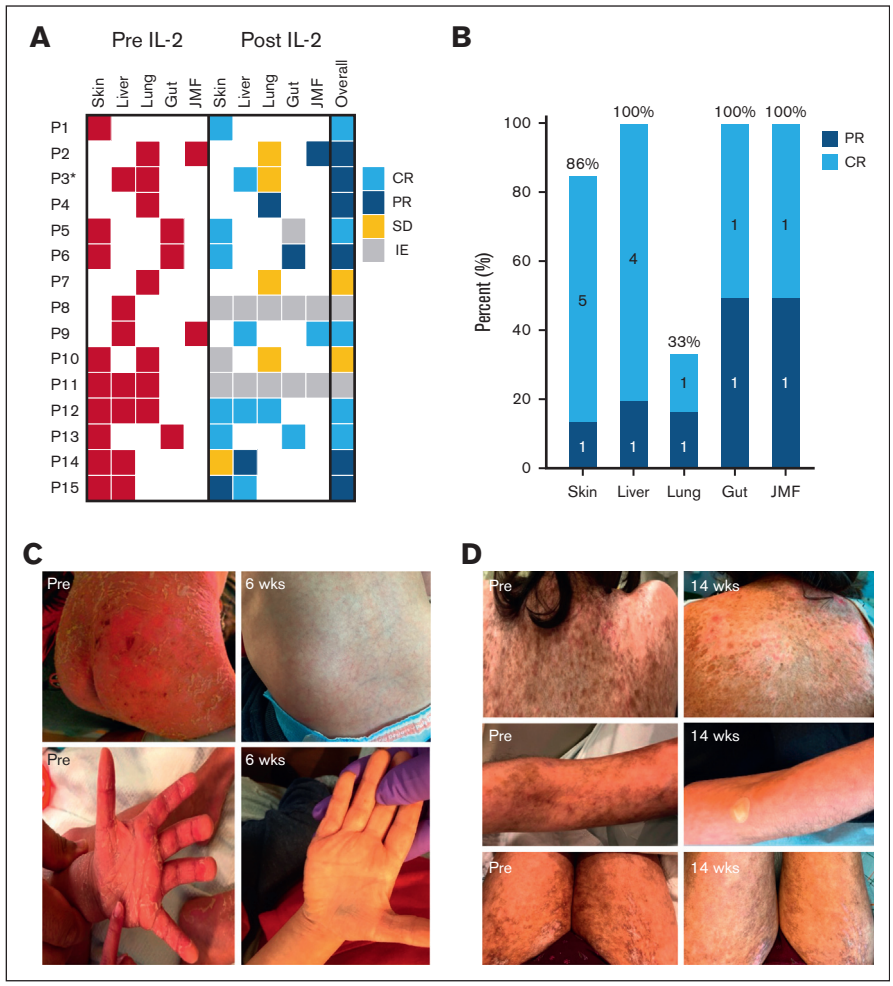

Figure 2. Clinical responses to LD IL-2 therapy. (A) Heatmap showing individual clinical responses for patients on a per-organ basis. (B) Summary of organ-specific clinical responses to LD IL-2 therapy. ORR is shown as percentage above bars. Number of patients with individual response (count) are embedded within each bar. (C) Improvement in erythroderma and desquamation after 6 weeks on therapy. (D) Improvement in scleroderma and hyperpigmentation at 14 weeks on therapy. *P3 initially started LD IL-2 for liver involvement and subsequently developed pulmonary cGVHD in the setting of pulmonary aspergillosis.

There were 2 deaths among the entire cohort of 15 patients with a median follow-up time among survivors of 22 months (range, 6-61 months). One-year survival was 86% (95% confidence interval, 53-96). Patient P8 was in liver failure from refractory liver cGVHD at the time of LD IL-2 initiation and also had ocular and oral involvement. He had no improvement of his ocular or oral cGVHD and died within 1 month of starting IL-2 therapy because of progressive liver failure. Patient P13 had a CR to LD IL-2 for skin and

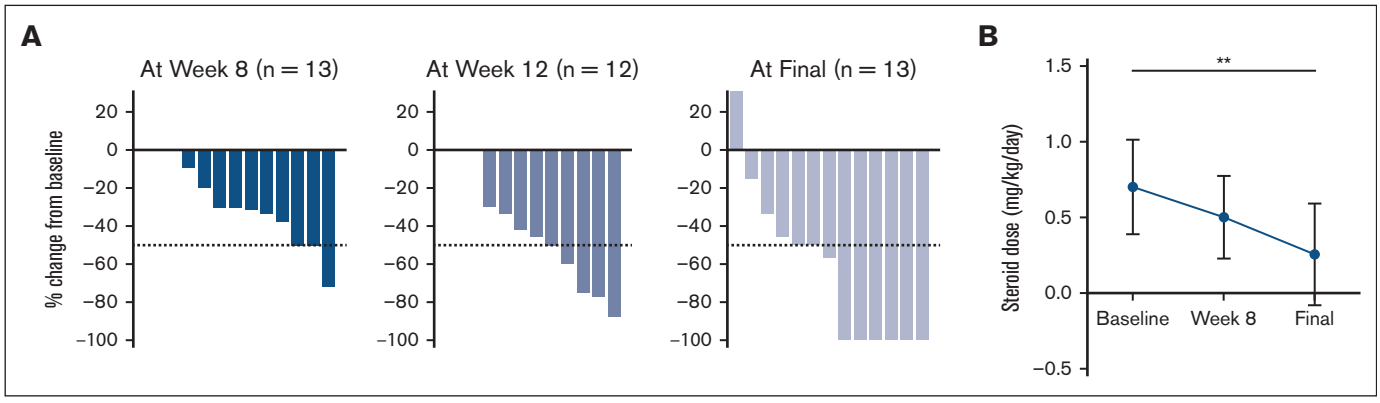

Figure 3. Steroid wean during LD IL-2 course. (A) Waterfall plots show decrease in steroid dose over time as compared with the dose at time of LD IL-2 start. (B) Steroid dose in mg/kg per day while on LD IL-2 therapy shown as mean with standard deviation in patients who were evaluable (n = 13). Baseline refers to steroid dose at time of LD IL-2 initiation. Final refers to steroid dose at time of LD IL-2 discontinuation. $^{**}P < .01$.

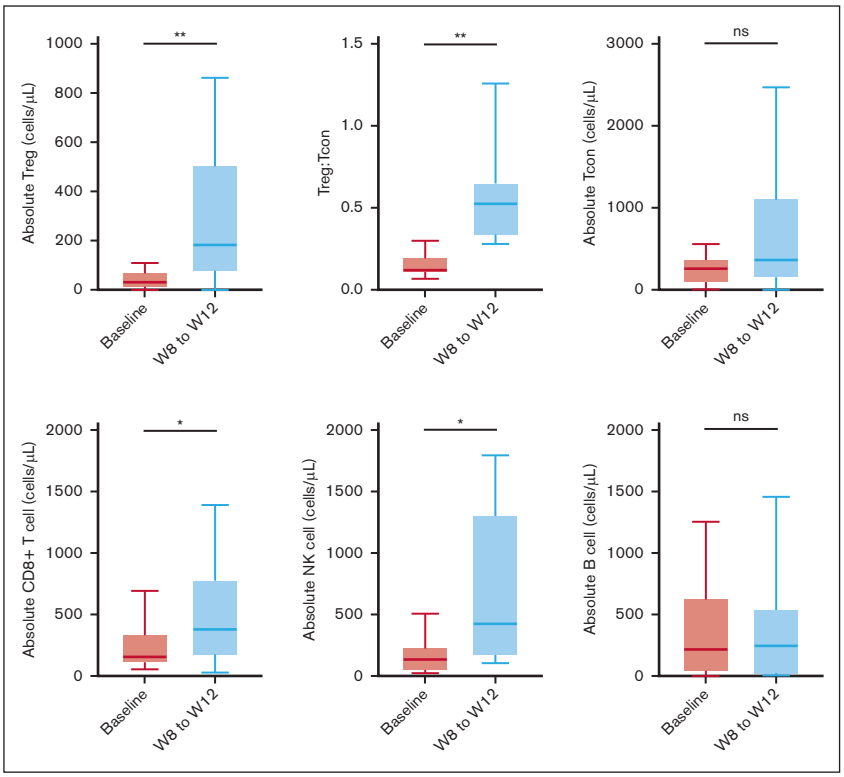

Figure 4. Immune cell responses to LD IL-2 therapy. Absolute cell counts for lymphocyte subsets including $CD4^+$ Tregs, $CD4^+$ Tcons, $CD8^+$ T cells, NK cells, and B cells as well as Treg:Tcon ratio are shown at baseline and after 8 to 12 weeks of LD IL-2 therapy. If patients had both week 8 (W8) and week 12 (W12) values, these were averaged. Baseline refers to the value at the measurement directly before LD IL-2 was initiated. $*P < .05$, $**P < .01$, $***P < .001$.

gut cGVHD but subsequently died from infection due to prolonged neutropenia in the setting of persistent human herpesvirus 6 viremia and poor graft function.

Clinical laboratory parameters

Although transient thrombocytopenia has been reported in previous studies of LD IL-2, IL-2–related cytopenias were not observed in this cohort. P11 stopped therapy after 8 days because of grade 4 thrombocytopenia but this was later determined to be from immune thrombocytopenia. As observed in prior LD IL-2 trials, eosinophilia, defined as an absolute eosinophil count of >1000/μL, was seen in several patients (supplemental Figure 2A). There were no clear clinical consequences of the eosinophilia, and there were no associated features of a systemic drug reaction. Eosinophilia resolved upon IL-2 discontinuation (supplemental Figure 2B).

Treg expansion

Because patients were not on a clinical trial, Treg numbers and other immune cell subsets were not measured consistently. However, lymphocyte subset data, performed over time in the clinical laboratory at Boston Children's Hospital, were available in the medical record for 9 patients (Figure 4). As expected, there was preferential expansion of Tregs over conventional T cells (Tcons). The median Treg:Tcon ratio was 0.12 (range, 0.07-0.30) at baseline and increased to 0.52 (range, 0.28-1.26) at week 8 to 12 ($P = 0.0039$) (supplemental Table 2). The median peak fold increase in the Treg:Tcon ratio over baseline was 2.8 (range, 2-19.8), which occurred by 8 weeks on therapy (supplemental Table 3). Interestingly, patients receiving concurrent ruxolitinib (n = 3 with lymphocyte subsets) and calcineurin inhibitors (n = 3 with lymphocyte subsets) showed similar Treg expansion to patients not on these therapies (supplemental Table 3). Similarly, there was no clear impact of the initial steroid dose on Treg expansion (P5 and P10 started LD IL-2 while on 1mg/kg per day steroids and had peak Treg:Tcon fold expansions of 19.8 and 2.8, respectively). The Treg:Tcon ratio increased in all patients on LD IL-2 therapy (supplemental Figure 3) but numbers were insufficient to assess correlation with clinical response. NK cells also express the high-affinity IL-2 receptor and expand in response to LD IL-2.[21] In this cohort, the NK cell population showed expected expansion on therapy with a median cell count per microliter at baseline of 134 (range, 25-507 cells per μL) compared with 424 cells per μL (range, 106.5-1794 cells per μL) at week 8 to 12 ($P = 0.012$; supplemental Table 2). The $CD8^+$ T-cell fraction also increased from a median cell count per microliter at baseline of 156 (range, 58-694 cells per μL) compared with 378 (range, 31-1390 cells per μL) at week 8 to 12 ($P = 0.027$). There was no significant increase in B-cell count by week 8 to 12.

Discussion

LD IL-2 is a safe and well-tolerated therapy with promising efficacy for cGVHD and autoimmune disease.[13-18,22-25] Although FDA-approved at high doses for renal cell carcinoma and metastatic melanoma since the 1990s, recombinant IL-2 is not yet approved at low doses for the promotion of immune tolerance in auto- and allo-immunity. Thus, use for these indications, to date, has been restricted to the clinical trial setting, almost exclusively in adult patients. Although clinical trials are well-controlled settings that allow for standardized assessment and correlative studies, they are

less permissive to the practical aspects of therapy such as dose adjustments, changing concomitant therapies, and intermittent disruptions of therapy. Our study reports the first off-study clinical experience using LD IL-2. We also build on our center's unique experience using LD IL-2 in pediatric patients with refractory cGVHD, including patients as young as 1.2 years of age.

As with our previous pediatric trial experience, we found that LD IL-2 is well tolerated, with the majority of patients continuing daily subcutaneous injections for >1 year. In addition, we observed a high ORR in pediatric patients with cGVHD that had failed multiple lines of prior therapy. All patients were started on our previously determined maximum tolerated dose of 1×10^6 IU/m^2 per day, which was sustained daily for the entire duration of therapy with a few exceptions. Three patients electively reduced their dose because of malaise or discomfort. Despite these alternate dosing regimens, the ORR in our real-world study was 85%, which is similar to the response rate observed in our previous phase 1 dose-escalation study in children (ORR, 82%; n = 11).[18] Furthermore, the 2 nonresponders in this off-study cohort were not patients for whom LD IL-2 doses had been reduced. As with this previous study, PR was seen in most patients by 8 weeks on therapy, and several patients attained organ-specific CR with extended-duration therapy (ie, >8 weeks), particularly for cutaneous and liver manifestations of cGVHD. Although steroid weaning practices are not standardized and do not always reflect improvement of cGVHD, almost all patients in this cohort were able to reduce their steroid dose without experiencing a cGVHD flare, including 6 patients who were able to discontinue altogether. Limiting steroid exposure is important for all patients, but it is particularly important in children to permit healthy growth, development, and metabolism. Overall, we recapitulated our previous on-study response rate despite dose reduction or alternate day dosing regimens for several patients. Thus, regimens other than daily dosing for LD IL-2 may also be efficacious.

Different dosing regimens of LD IL-2 have also been used for indications other than cGVHD. Our daily dosing regimen, while still relatively "low dose," is notably higher compared with another published report of LD IL-2 in a phase 1/2 trial for pediatric patients with type I diabetes.[22] In that study, IL-2 was given for 5 days and then once every 2 weeks for a year, with the highest dose being 0.5×10^6 IU/m^2. There were no serious adverse events, and there was a dose-dependent correlation with Treg expansion, with "Treg-high" responders having more preserved insulin production at 1 year. This strategy of more frequent dosing during an induction period followed by less frequent maintenance dosing has also been used in adult trials for systemic lupus erythematosus, which have shown that it is clinically well tolerated, expands the Treg fraction, and has early suggestions of efficacy.[24,25] Larger studies with pharmacokinetic analysis of serum IL-2 levels will be needed to determine the optimal dosing regimen.

As expected, LD IL-2 preferentially expanded the Treg fraction resulting in a higher Treg:Tcon ratio in the subset of patients with available immune cell values in the medical record. Beyond increasing the absolute number of Tregs and the Treg:Tcon ratio, LD IL-2 is also thought to have a beneficial effect on Treg diversity and function.[16,18] Previous work at our center demonstrated that LD IL-2 increases Treg T-cell receptor β clonotype diversity and that this corresponds to better clinical response. In a study of LD IL-2 for systemic lupus erythematosus, the expression of 55 different genes was upregulated in Tregs after 2 months on treatment when compared with baseline.[26] These differentially expressed genes were associated with cellular activation and tissue homing, including an HLA-DR$^+$ subset of Tregs showing homing toward the skin based on biopsy data.[26] Although similar work has not been performed in cGVHD, this subpopulation of cells could explain why LD IL-2 appears to be particularly effective for skin cGVHD, including reversal of sclerotic skin as observed in 2 patients in our off-study cohort.

Lastly, because patients could be on multiple concomitant therapies, we explored whether cotherapies might impair Treg expansion from LD IL-2. This was not seen, as high starting steroid dose (median 0.79 mg/kg per day) or the presence of ruxolitinib or calcineurin inhibitors did not appear to impair Treg expansion (or clinical response for those without laboratory data). Ruxolitinib is of interest because the IL-2 receptor is a JAK 1/3 receptor. Although ruxolitinib is more specific for JAK 1/2-based cytokine receptors, there could theoretically be nonspecific blockade that could dampen the therapeutic effect of LD IL-2.[27] This was not reflected in our small cohort, as 5 patients were concurrently receiving ruxolitinib at time of LD IL-2 start and 4 had objective clinical responses (1 inevaluable) with Treg expansion in all 3 patients with available immune cell data. Calcineurin inhibitors block IL-2 production by effector T cells, which may be detrimental to Tregs by eliminating their critical source of IL-2. However, by providing the IL-2 exogenously, this problem is overcome, and Tregs can still expand while on concurrent LD IL-2 therapy and calcineurin inhibition.[28] The ability for LD IL-2 to pair well with other drugs makes it attractive for clinical use, although our findings require further validation with higher patient numbers.

Although we are encouraged by our collective pediatric data, which now includes 24 pediatric patients with multiple years of follow-up, it has become increasingly difficult to obtain insurance approval for LD IL-2 because new small molecules have achieved FDA approval for second-line therapy of cGVHD. While our results reflect only single-center experience, LD IL-2 has consistently been shown as a safe and effective therapy with 105 adult and 11 pediatric patients[29] treated across 5 clinical trials with efficacy at least on par with the FDA-approved agents. It is our hope that a Children's Oncology Group–sponsored trial of LD IL-2 for refractory cGVHD in children, currently in development (Carrie Kitko, personal communication, 30 March 2023), can help to establish efficacy in a multicenter setting and subsequently advance the approval status of LD IL-2 for this indication.

Additional work with larger patient numbers is needed to determine baseline predictive factors for response to IL-2 and mechanisms of clinical improvement in different organs. Currently, young age and cutaneous or liver cGVHD involvement appear to be reasonable clinical predictors (the latter 2 for an organ-specific response) in pediatric patients, which may differ from the adult experience in which there was a lower cutaneous response.[29] This may be because of underlying age-related differences in thymic output and cGVHD biology. Our center has also shown that combination therapy of LD IL-2 with *ex vivo* expanded Tregs can promote persistence of adoptively transferred Tregs in a nonhuman primate model, which is serving as the impetus for an upcoming clinical trial.[30] An advantage of an exogenous Treg therapy is that it is

possible to engineer even greater IL-2 specificity by using orthogonal IL-2/IL-2 receptor pairs.[31,32] However, even for IL-2 biologic monotherapy, efforts are being made to engineer IL-2 muteins that have a longer half-life while being selective for the high affinity IL-2 receptor.[33,34] Although we have not yet identified any clinical adverse effects from expansion of non-Treg cell populations, further enhancing specificity might limit epiphenomena such as the eosinophilia observed in many patients on LD IL-2, which is thought to be due to the release of IL-5 from IL-2 exposed innate lymphoid cells.[35]

In summary, daily subcutaneous LD IL-2 is safe and well tolerated in children as young as 1.2 years of age for years on therapy. The majority of children have at least a PR, which is generally detectable by 8 weeks. Responses for liver and skin cGVHD are particularly notable, with most patients achieving CR in these organs. Our study thus supports the use of LD IL-2 in SR-GVHD, which has a high clinical unmet need. In addition, these data make a compelling case for further exploring the use of LD IL-2 to promote immune tolerance in pediatric patients with autoimmune disease or after solid organ transplantation.

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Authorship

Contribution: H.W., M.K., L.S.K., J.R., J.K., and J.S.W. conceived and designed the study; F.A.C., S.B., C.D., S.F., L.G., J.H., L.E.L., H.L., and M.S. referred/recruited patients; H.W., M.K., J.H., and J.S.W. collected and assembled the data; H.T.K. analyzed and interpreted the data, and performed statistical analysis; and all authors wrote the manuscript.

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