Association of Serum Interleukin-7 Levels With the Development of Acute Graft-Versus-Host Disease

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ABSTRACT

Purpose

Morbidity from acute graft-versus-host disease (GVHD) limits the success of allogeneic hematopoietic stem-cell transplantation (HSCT) to treat malignancy. Interleukin-7 (IL-7), the principal homeostatic cytokine for T cells, is required for acute GVHD in murine models. In contrast to inflammatory cytokines (eg, IL-2, tumor necrosis factor α), IL-7 has not been studied extensively in the clinical transplant setting relative to its relationship with acute GVHD.

Patients and Methods

We evaluated the association of serum IL-7 levels with acute GVHD in 31 patients who were uniformly treated in a prospective clinical trial with reduced-intensity allogeneic HSCT from human leukocyte antigen-identical siblings. GVHD prophylaxis consisted of cyclosporine and methotrexate. Serum IL-7 levels and lymphocyte populations were determined at enrollment, the day of transplantation before the allograft infusion, and at specified intervals through 12 months post-transplantation.

Results

As expected, IL-7 levels were inversely correlated with T-cell populations ($P < .00001$). Acute GVHD was significantly associated with higher IL-7 levels at day +7 ($P = .01$) and day +14 ($P = .00003$) post-transplantation as well as with the allograft CD34⁺ cell dose ($P = .01$). IL-7 levels at day +14 also correlated with the severity of acute GVHD ($P < .0001$). In logistic regression models, these factors were highly sensitive (up to 86%) and specific (100%) for classifying whether patients developed acute GVHD.

Conclusion

These data support preclinical observations that IL-7 plays a critical role in inducing acute GVHD and provide a rational basis for novel approaches to prevent and treat acute GVHD through modulation of the IL-7 pathway.

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INTRODUCTION

The morbidity that may result from acute graft-versus-host disease (GVHD) poses a major barrier to the successful application of allogeneic hematopoietic stem-cell transplantation (HSCT) to treat malignancy.¹ On infusion, the allogeneic effector T-cell populations that initiate acute GVHD are exposed to elevated levels of various cytokines, of which many are inflammatory mediators, (eg, tumor necrosis factor α) whose contribution to acute GVHD has been the focus of extensive prior study.²

Allogeneic effector T cells are also directly influenced by homeostatic cytokines, particularly interleukin-7 (IL-7), that promote immune reconstitution after allogeneic HSCT. As the principal T-cell homeostatic cytokine, IL-7 is critical for T-cell development and survival.³ IL-7 is produced consti-

tutively by stromal cells and consumed by the available pool of resting T cells, all of which express the IL-7 receptor (IL-7R) at high levels except for CD4⁺CD25⁺ regulatory T cells.⁴ Systemic IL-7 levels increase during periods of lymphopenia to maintain naïve T-cell homeostasis and support the thymic-independent peripheral expansion and maintenance of mature T cells.⁵⁻⁷ The conditioning regimen administered before allogeneic HSCT causes severe host lymphopenia, thus perturbing T-cell homeostasis and creating a milieu in which infused allogeneic lymphocytes are exposed to elevated levels of endogenous IL-7.⁵ These conditions promote the expansion and persistence of allogeneic T cells that have the potential to recognize host antigens, become activated, and initiate GVHD.

Murine models from our laboratory and others implicate IL-7 in the development of acute

GVHD.⁸⁻¹¹ However, a relationship between IL-7 and acute GVHD has not been identified in the limited clinical data that are published to date.¹² We evaluated the association of IL-7 levels with the development of acute GVHD in a prospective clinical trial, finding that serum IL-7 levels in the early post-transplantation period were sensitive and specific for predicting the development of acute GVHD.

PATIENTS AND METHODS

Patients

Patients were eligible for this study if they had a high-risk hematologic malignancy and a consenting human leukocyte antigen–matched sibling donor. The study (CC# 03-C-0077) was approved by the National Cancer Institute Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Treatment

Before the transplant conditioning regimen, all patients received an identical, conventional-dose induction regimen with the goal of achieving a relatively homogeneous patient population with respect to host T-cell lymphopenia. Up to three cycles of this regimen, consisting of etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and fludarabine (EPOCH-F), were administered until the peripheral-blood CD4⁺ T-cell count was less than 100 cells/ μ L.¹³ Patients with CD20⁺ malignancies also received rituximab with EPOCH-F. All patients then received reduced-intensity conditioning consisting of fludarabine (30 mg/m²/d) and cyclophosphamide (1,200 mg/m²/d) for 4 days. Donor peripheral-blood hematopoietic progenitor cells were mobilized with filgrastim, collected by apheresis, and cryopreserved until the day of transplantation. GVHD prophylaxis consisted of cyclosporine and methotrexate. Cyclosporine was administered twice daily, starting the day before transplantation, maintained at serum levels between 200 and 250 ng/mL through 3 months post-transplantation, then tapered to discontinuation by 6 months post-transplantation in the absence of GVHD. Methotrexate (5 mg/m²) was administered on days +1, +3, +6, and +11 after transplantation. Acute GVHD was confirmed by biopsy of at least one involved site and graded according to the 1994 Consensus Criteria.¹⁴

Serum IL-7 Enzyme-Linked Immunosorbent Assay

Serum samples for IL-7 determination were obtained at enrollment before EPOCH-F induction chemotherapy; on the day of transplantation before infusion of the allograft; and at day +7, day +14, 1 month, 2 months, 3 months, 6 months, 9 months, and 12 months post-transplantation. Sera were prepared by centrifugation of blood and stored in 0.5-mL aliquots at –80°C. Samples were analyzed using a high-sensitivity colorimetric enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). Along with patient serum samples, each ELISA plate was loaded with serial dilutions of a standard recombinant IL-7 solution that was provided with the ELISA assay, according to the manufacturer's instructions. The results of these samples were used to prepare standard curves for each plate. Patient samples whose IL-7 levels exceeded the range defined by the standard samples were diluted and rerun on another ELISA plate. Plates were analyzed with a microplate reader and SOFTmax Pro 3.0 software (Molecular Devices Corporation, Sunnyvale, CA).

Flow Cytometry

Blood was collected at the same time points as serum IL-7 measurements. Flow cytometric enumeration of lymphocyte populations was performed using whole-blood lysis by a Clinical Laboratory Improvement Amendments–certified laboratory (Science Applications International Corporation, Frederick, MD). The absolute numbers of CD3⁺, CD4⁺CD3⁺ and CD8⁺CD3⁺ T cells, and CD3⁺CD56⁺ NK cells were calculated using the percentages of these cells in the lymphocyte gate and the absolute lymphocyte count from a CBC (Beckman Coulter Inc, Fullerton, CA).

Donor-Recipient Hematopoietic Chimerism

Chimerism analysis was performed at day +14, 1 month, and 3 months post-transplantation on total peripheral-blood mononuclear cells, using the variable number tandem repeats–polymerase chain reaction method in a Clinical Laboratory Improvement Amendments–certified laboratory (Blood Center of Southeastern Wisconsin, Milwaukee, WI). Chimerism was also determined on blood samples enriched for myeloid (CD15⁺ or CD33⁺) and T-lymphoid (CD3⁺) subsets.

Table 1. Characteristics of Patients Undergoing Reduced-Intensity Allogeneic Hematopoietic Stem-Cell Transplantation

Characteristic	Value
Age, years	
Recipient	
Median	57
Range	31-71
Donor	
Median	50
Range	29-74
Diagnosis, No.	
Non-Hodgkin's lymphoma	17
CLL/SLL	4
PLL	
Hodgkin's lymphoma	5*
MDS/AML	3
MPD	2
Disease status at transplantation, No.	
Complete remission	6
Partial remission	6
Stable disease	14
Progressive disease	4
Not assessable	1
CD20 ⁺ malignancy (rituximab exposure), No.	
Yes	18
No	13
Sex (donor/recipient), No.	
Male/male	9
Male/female	3
Female/male	15
Female/female	4
Donor pregnancy, No.	
Yes	15
No	1
Unknown	3
CMV status (donor/recipient), No.	
Negative/negative	9
Negative/positive	3
Positive/negative	4
Positive/positive	15
Allograft composition	
CD34 ⁺ cells/kg recipient weight ($\times 10^6$)	
Median	6.8
Range	4.0-17.4
CD3 ⁺ cells/kg recipient weight ($\times 10^9$)	
Median	3.2
Range	1.0-5.3

Abbreviations: CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; PLL, prolymphocytic leukemia; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; MPD, myeloproliferative disorder; CMV, cytomegalovirus.

*One prior follicular non-Hodgkin's lymphoma.

Statistical Analysis

Overall survival (OS), progression-free survival (PFS), and the proportions of patients developing acute and chronic GVHD were estimated using the Kaplan-Meier method. Serum IL-7 levels and peripheral-blood lymphocyte counts were compared with dichotomous clinical parameters including acute GVHD; chronic GVHD; engraftment syndrome requiring corticosteroid administration, defined as noninfectious fever, erythematous rash, and noncardiogenic pulmonary edema resulting in diffuse pulmonary infiltrates and hypoxemia, occurring within 96 hours of neutrophil recovery;¹⁵ full donor chimerism, defined as at least 95% donor origin for all cell populations measured, at day +14, 1 month, and 3 months post-transplantation; and cytomegalovirus infection. Other relevant clinical and immunologic parameters were analyzed for potential associations with acute GVHD, as shown in Table 4. Comparisons employed the exact Wilcoxon rank sum tests for continuous parameters and Fisher's exact test for categorical parameters. Logistic regression analysis was used to determine which parameters identified as potentially significant in the univariate analysis were jointly associated with acute GVHD. Correlation analyses used Spearman (nonparametric) analysis, with r less than 0.70 indicating strong correlations and r between -0.5 and -0.7 indicating moderately strong correlations. All P values are two tailed and have not been formally adjusted for multiple comparisons; however, in this exploratory study, P values less than .005 would be readily interpretable as being statistically significant whereas those such that P between .005 and .05 would reflect strong trends.

RESULTS

Between January 2003 and August 2004, 31 patients were enrolled (Table 1). The median recipient age was 57 years. Donor-recipient sex mismatch was present for 18 recipients. In nine cases, both the donor and the recipient were seronegative for cytomegalovirus; 22 recipients were either seropositive themselves ($n = 18$) and/or had a seropositive donor ($n = 17$). Disease status at the time of transplantation was chemotherapy-sensitive (complete or partial remission) in 12 patients, chemotherapy-resistant (stable or progressive disease) in 18 patients, and not assessable in one patient.

Transplant Outcomes

Infused allografts contained a median 6.8×10^6 CD34⁺ cells/kg and 3.2×10^8 CD3⁺ cells/kg. Median donor T-cell chimerism at day +14 post-transplantation was 98% (range, 60% to 100%), with full

donor T-cell chimerism in 23 patients (74%); median donor mononuclear cell chimerism at 1 month and 3 months was 100% (range, 55% to 100%) and 100% (range, 10% to 100%), respectively, with full donor mononuclear cell chimerism in 24 patients (77%) and 22 patients (71%), respectively. There were no primary graft failures.

Grades 1 to 3 acute GVHD (Table 2) occurred in 14 (45%) of 31 patients, with grades 2 to 3 occurring in 13 patients (42%). One case of grade 1 acute GVHD occurred with isolated stage 2 skin involvement. No grade 4 acute GVHD was observed. Grade 2 acute GVHD was predominantly cutaneous; five of six patients with grade 3 acute GVHD had GI involvement. Median time to onset of acute GVHD was 33 days (range, 19 to 49 days) post-transplantation. Chronic GVHD occurred in 21 of 29 assessable patients, of whom five have died. At 2 years post-transplantation, chronic GVHD had occurred in 72% of patients. Two patients who died as a result of progressive

Fig 1. (A) Median serum interleukin-7 (IL-7) levels and blood lymphocyte counts from baseline through 12 months post-transplantation. (B) Median serum IL-7 levels according to subsequent development of acute graft-versus-host disease (aGVHD). Error bars denote interquartile ranges. ALC, absolute lymphocyte count.

Table 2. Relationship of Acute and Chronic GVHD to Serum IL-7 Levels

GVHD	No. of Patients	%	Organ Involvement (No. of patients)			Median IL-7 Level (pg/mL)	
			Skin	Gut	Liver	Day +7	Day +14
Acute							
None	17	55				11.8	8.2
Grade I	1	3	1	0	0	23.9	14.0
Grade II	7	23	6	3	0	32.8	23.0
Grade III	6	19	3	5	2	25.7	27.8
Grade IV	0	0					
Chronic							
Yes	21	68				12.82	12.42
No	8	26				24.85	12.27
Not assessable	2	6				—	—

Abbreviations: GVHD, graft-versus-host disease; IL, interleukin.

Table 3. Correlation of IL-7 Levels With Lymphocyte Counts Over Time

Level	Baseline	Day 0	Day +7	Day +14	1 Month	2 Months	3 Months	6 Months	9 Months	12 Months
IL-7, pg/mL										
Median	12.1	37.0	13.5	12.0	9.4	6.5	7.6	9.8	9.0	15.0
Range	0-46.9	13.3-79.2	3.0-73.0	1.3-74.7	1.3-34.9	1.5-70.0	0.2-68.0	0-43.5	0-71.2	3.2-33.7
ALC, cells/ μ L										
Median	871	3	76	493	1,138	1,028	1,014	1,298	1,600	1,220
Range	95-43,817	0-322	6-525	9-2,904	149-3,172	145-5,564	110-3,606	147-4,140	207-3,909	229-5,494
<i>R</i>	−0.49	−0.28	−0.83	−0.35	−0.25	−0.22	−0.08	−0.24	−0.40	−0.22
<i>P</i>	.008	.14	< .0001	.06	.18	.27	.66	.30	.06	.36
CD3, cells/ μ L										
Median	629	3	60	307	773	777	745	1003	1339	985
Range	5-2,858	0-69	5-464	6-1,428	128-2,927	112-5,369	97-3,516	95-3,908	172-3,729	200-5,257
<i>R</i>	−0.52	−0.28	−0.82	−0.36	−0.39	−0.22	−0.14	−0.25	−0.38	−0.33
<i>P</i>	.005	.14	< .0001	.05	.04	.28	.47	.29	.07	.15
CD4, cells/ μ L										
Median	294	3	45	206	405	310	352	439	494	440
Range	5-1,652	0-65	5-385	5-810	75-1,145	78-901	40-1,493	35-1,457	70-1,176	33-1,032
<i>R</i>	−0.58	−0.22	−0.86	−0.33	−0.36	−0.19	−0.08	−0.34	−0.20	−0.25
<i>P</i>	.001	.24	< .0001	.07	.05	.36	.66	.14	.34	.29
CD8, cells/ μ L										
Median	269	0	16	95	326	364	356	557	660	397
Range	1-1,763	0-22	0-88	1-963	27-2,123	31-4,484	57-2,990	57-2,447	98-2,932	165-4,258
<i>R</i>	−0.54	−0.35	−0.65	−0.48	−0.32	−0.22	−0.16	−0.22	−0.46	−0.22
<i>P</i>	.003	.06	.0002	.006	.09	.27	.39	.36	.02	.34

NOTE. Correlation coefficients and *P* values shown are for comparison with interleukin-7 (IL-7) levels at specified time points.
Abbreviation: ALC, absolute lymphocyte count.

lymphoma at days +125 and +166 post-transplantation, respectively, were not assessable for chronic GVHD. With a median potential follow-up of 26.7 months, actuarial PFS and OS probabilities at 2 years were 49.5% (median, 20.6 months) and 64% (median not reached), respectively.

Serum IL-7 Levels and Peripheral-Blood Lymphocyte Counts

Median IL-7 levels rose from 12.1 pg/mL (range, 0 to 46.9 pg/mL) at enrollment to a peak of 37 pg/mL (range, 13.3 to 79.2 pg/mL) on day 0 after the transplant conditioning regimen, but before the infusion of the allograft (Fig 1A; Table 3). Median IL-7 levels subsequently declined to 12.0 pg/mL (range, 1.3 to 74.7 pg/mL) by day +14 after transplantation and remained relatively stable thereafter. Inverse correlations were present between serum IL-7 levels and circulating CD3⁺ T-cell counts at baseline ($r = -0.52$; $P = .005$), and at day +7 post-transplantation ($r = -0.82$; $P < .0001$). Absolute lymphocyte, CD4, and CD8 counts were also inversely correlated with serum IL-7 levels at day +7 (Table 3).

IL-7 and Acute GVHD

The IL-7 levels at day +7 ($P = .01$) and day +14 ($P = .00003$) post-transplantation were associated with the subsequent development of acute GVHD (Table 4; Fig 1B). Among patients who developed acute GVHD, median IL-7 levels were 25.7 and 20.9 pg/mL at days +7 and +14, respectively. Patients who did not develop acute GVHD had median IL-7 levels of 11.8 and 8.22 pg/mL at days +7 and +14, respectively. Higher IL-7 levels at day +14 were strongly associated with more severe grades of acute GVHD ($P < .0001$; Table 2).

Among other clinical parameters (Table 4), only the allograft CD34⁺ cell dose was also strongly ($P = .01$) associated with acute GVHD. Acute GVHD was not associated with prior rituximab administration ($P = .72$). The achievement of complete donor myeloid or lymphoid chimerism by day +14 post-transplantation was not associated with the development of acute GVHD. Serum IL-7 levels were not significantly associated with full donor chimerism through 3 months post-transplantation (data not shown) or with the development of chronic GVHD. IL-7 levels at day +7 or day +14 post-transplantation demonstrated no significant association with PFS or OS, although there was a modest trend toward better OS among patients with serum IL-7 levels less than 24.4 pg/mL at day +7 ($P = .07$). Disease status at transplantation was not associated with either IL-7 levels (data not shown) or the development of acute GVHD ($P = .71$).

Logistic regression analysis confirmed that the IL-7 level at day +14 was the strongest single parameter associated with acute GVHD. Using a cutoff value of 13 pg/mL, the IL-7 level at day +14 predicted the subsequent development of acute GVHD with a sensitivity of 85.7% and specificity of 88.2%. The positive and negative predictive values for this single-variable model were 85.7% and 88.2%, respectively. Alternate predictive models that included the CD34⁺ cell dose and the IL-7 level at day +7 were also evaluated. The close correlation between IL-7 levels at day +7 and day +14 ($r = 0.69$; $P < .0001$) precluded both parameters from appearing jointly in a model. IL-7 at day +7 alone was less accurate than IL-7 at day +14 alone (data not shown). When the CD34⁺ cell dose was included with the IL-7 level at either day +7 or day +14, the resulting models predicted acute GVHD with only two errors (Tables 5 and 6), with sensitivity of 84.6% or 85.7%, respectively, and with 100% specificity for either model.

Table 4. Results From Univariate Analysis of Factors for Acute GVHD

Variable	Acute GVHD		P
	Yes	No	
Patient age, years			.91
Median	57	53	
Range	32-71	31-63	
Donor age, years			.18
Median	53	48	
Range	38-74	29-67	
Disease status at transplant*			
CR or PR	6	7	.71
SD or PD	6	11	
Graft CD3 dose, $\times 10^6$ cells/kg			.27
Median	284	349	
Range	176-610	103-533	
Graft CD34 dose, $\times 10^6$ cells/kg			.01
Median	9.15	5.72	
Range	3.95-17.40	4.0-10.71	
IL-7 at day +7, pg/mL			.01
Median	25.7	11.8	
Range	7.3-73.0	3.0-27.8	
IL-7 at day +14, pg/mL			.00003
Median	20.9	8.2	
Range	9.9-74.7	1.3-27.2	
Donor sex			.99
Male	5	7	
Female	9	10	
Sex mismatch			.99
Yes	8	10	
No	6	7	
CMV exposure			.46
Yes	11	11	
No	3	6	
Prior rituximab			.72
Yes	9	9	
No	5	8	
Donor chimerism at day +14			
Myeloid > 95%			.67
Yes	10	12	
No	2	4	
Lymphoid > 95%			.99
Yes	10	13	
No	4	4	

Abbreviations: GVHD, graft-versus-host disease; IL, interleukin; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease; CMV, cytomegalovirus.

*Disease status was not assessable for one patient.

Because engraftment syndrome (ES) may result from alloreactivity after allogeneic HSCT,¹⁵ the relationship between IL-7 at day +14 and acute GVHD was re-examined with all cases of ES reclassified as acute GVHD, finding a similarly strong association between IL-7 at day +14 and acute GVHD in univariate analysis ($P = .00001$).

DISCUSSION

Multiple cytokines contribute to the initiation, severity, and persistence of acute GVHD.² Prior studies have mainly focused on inflam-

matory cytokines; however, murine models indicate that IL-7 is necessary for the initiation of GVHD.^{10,11} We prospectively studied patients who underwent allogeneic HSCT in a uniform manner to generate preliminary clinical data relative to the relationship between serum IL-7 levels and acute GVHD. These data demonstrated that higher serum IL-7 levels in the early post-transplantation period were strongly associated with both the subsequent development and the severity of acute GVHD. The magnitude of the effect that we observed, even with a relatively small number of patients, supports the validity of these data and is consistent with the central role of IL-7 in T-cell homeostasis.

Our study confirms that serum IL-7 levels correlate inversely with the numbers of circulating T cells. The patients in this study received induction chemotherapy before reduced-intensity conditioning to produce deliberate host lymphodepletion. This design helped to clarify the relationship of IL-7 to acute GVHD by minimizing the influence of other possible confounding factors. First, host T cells were uniformly depleted to levels that are comparable with those achieved with myeloablative conditioning regimens, thus potentiating the homeostatic induction of IL-7 in the peritransplantation period. Second, unlike some nonmyeloablative regimens, this reduced-intensity platform brought about complete donor chimerism in most patients in the early post-transplantation period. This is relevant because states of mixed donor chimerism are associated with a decreased incidence of acute GVHD,¹⁶ although no significant relationship was detected between chimerism and GVHD in our analysis.

The CD34⁺ cell dose was the only other parameter that demonstrated a significant association with acute GVHD in univariable and multivariable analyses. Stem-cell dose has been identified as a risk factor for acute GVHD in some studies, although the physiologic basis for this relationship is undefined.^{17,18} In our analysis, CD34⁺ cell dose had a modest impact on the risk of acute GVHD, compared with the effect of serum IL-7 levels. However, there is evidence that IL-7 can influence CD34⁺ T-cell precursors,^{19,20} and a hypothesis that might explain our findings is that higher IL-7 levels potentiated T-cell proliferation and maturation among patients with higher CD34⁺ cell doses.

Several patients on our trial developed ES. The biology of ES is poorly defined, and its distinction from acute GVHD is somewhat controversial. When we re-examined the effect of IL-7 with all cases of ES reclassified as acute GVHD, the association between IL-7 and acute GVHD was strengthened. This interesting observation supports a potential biologic relationship between ES and acute GVHD after allogeneic HSCT.

Further studies are needed to define the mechanisms underlying our findings. One possible explanation is that elevated serum IL-7 levels are a biologic marker for patients who are in the preclinical stages of acute GVHD. T cells that bind antigen and undergo activation downregulate IL-7R, leading to reduced IL-7 consumption.²¹ Thus, more pronounced elevations in serum IL-7 levels might be expected in patients with incipient acute GVHD in whom alloreactive donor T cells have become activated. Such a phenomenon is consistent with the findings of a murine model of allogeneic HSCT, in which alloreactive T cells lacked IL-7R expression.²² However, less than 1% of the T-cell clones within a graft are potentially alloreactive,²³ so this mechanism may not adequately explain the relationship between IL-7 and acute GVHD.

Table 5. Logistic Regression Analysis for Development of Acute Graft-Versus-Host Disease

Model	Parameter Estimate	P	Odds Ratio	95% CI	No. of Patients
#1					
Intercept	−3.26	.006			31
IL-7 day +14	0.21	.01	1.24	1.04 to 1.46	
#2					
Intercept	−11.38	.01			29*
IL-7 day +7	0.25	.02	1.28	1.04 to 1.59	
CD34 ⁺ cell dose	0.88	.03	2.41	1.10 to 5.28	
#3					
Intercept	−6.90	.004			31
IL-7 day +14	0.18	.009	1.20	1.05 to 1.38	
CD34 ⁺ cell dose	0.50	.04	1.66	1.01 to 2.70	

NOTE. Intercept is a logistic regression term indicating a constant value in deriving the equation to determine the probability of the outcome of interest.

Abbreviation: IL, interleukin.

*IL-7 data missing at day +7 for two patients.

Another possible explanation is that higher IL-7 levels directly lead to increased incidence and severity of acute GVHD. IL-7 lowers the threshold to develop acute GVHD, given that signaling through both the T-cell receptor (TCR) and IL-7R simultaneously is synergistic for activation and expansion of T cells. In murine models, costimulation with recombinant human IL-7 (rhIL-7) and anti-TCR antibody significantly increased secretion of the inflammatory cytokine interferon- γ by spleen-derived T cells from experimental bone marrow transplant recipients, and administration of rhIL-7 after allogeneic HSCT increased the severity of GVHD in a donor T-cell-dependent manner, possibly by augmenting both effector cell number and reactivity towards subdominant antigen.^{8,24} IL-7 also prevents T cells from undergoing activation-induced cell death, and so may provide a critical survival signal for effector T cells that initiate and sustain acute GVHD. Alternatively, IL-7 enhances the development and function of dendritic cells,³ which would favor greater presentation of alloantigens after allogeneic HSCT.

Our observations have significant clinical implications. Early prediction of acute GVHD based on serum IL-7 levels as a validated biologic marker could allow an individualized, risk-adapted strategy for prophylaxis of acute GVHD. Other assays related to inflammatory cytokines have been evaluated to identify patients at risk for developing acute GVHD,²⁵⁻²⁹ but these have not achieved wide-

spread clinical use, possibly because of limitations concerning their predictive power, complexity, availability, or cost. In contrast, the approach used in this study employs technologies that are comparatively simple, inexpensive, and highly reproducible. If serum IL-7 levels directly influence the development of acute GVHD, then the IL-7 signaling pathway may provide novel targets for treatment and even prevention of acute GVHD.³⁰ Our findings also suggest potential pitfalls in clinical trials of rhIL-7 as an immune adjuvant after allogeneic HSCT, and it needs to be determined whether the association between IL-7 and acute GVHD in our study can be generalized to other conditions that modulate the risk of GVHD (eg, allograft T-cell depletion and conditioning regimen intensity) because murine studies indicate that the safety of rhIL-7 administration in this setting may depend on such factors.^{8,9,22,31} We are initiating a multi-institutional study of the association of IL-7 and acute GVHD in varying allogeneic HSCT settings that vary by conditioning regimen and stem-cell source.

In conclusion, the observations in this clinical study substantiate the findings of preclinical murine models and are consistent with prior knowledge regarding the biologic role of IL-7 in T-cell homeostasis and the pathogenesis of acute GVHD. Serum IL-7 levels quantify the risk of acute GVHD and may enable a highly individualized clinical approach to managing acute GVHD after allogeneic HSCT. The IL-7 pathway represents a promising target for the development of novel agents to prevent and treat acute GVHD.

Table 6. Classification Results by Model

Predicted	Observed aGVHD	
	Yes	No
Model 1		
Yes	12	2
No	2	15
Model 2		
Yes	11	0
No	2	16
Model 3		
Yes	12	0
No	2	17

Abbreviation: aGVHD, acute graft-versus-host disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Robert M. Dean, Terry Fry, Crystal Mackall, Seth Steinberg, Ronald Gress, Michael R. Bishop

Financial support: Michael R. Bishop

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