Prediction of outcomes after second-line treatment for acute graft-versus-host disease

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Key Points

- Point estimates of survival and FFS at 6 months from second-line treatment for acute GVHD were 64% and 46%, respectively.
- We identify risk factors and provide models to predict 6- and 12-month mortality after second-line systemic treatment for acute GVHD.

Acute graft-versus-host disease (GVHD) requiring second-line treatment represents a highly morbid complication of allogenic hematopoietic cell transplantation (HCT). Recent studies have defined short-term outcomes after second-line treatment for acute GVHD, but longer-term outcomes have not been well defined. We examined overall survival (OS) and failure-free-survival (FFS) of 216 patient who had HCT who received second-line treatment for acute GVHD. Failure time for FFS was defined as the earliest of death, relapse, or implementation of third-line treatment. Multivariable Cox regression was used to identify risk factors for mortality and failure, and predictive models were derived for 6- and 12-month mortality. Point estimates of OS at 6 and 12 months were 59% (95% confidence interval [CI], 52-65) and 52% (95% CI, 45-68), respectively. Point estimates of FFS at 6 and 12 months were 42% (95% CI, 35-48) and 37% (95% CI, 31-43), respectively. Predictive models for both end points included serum albumin and total bilirubin concentrations at the onset of second-line treatment, patient age at onset of second-line therapy, and a combination of abdominal pain/stage 4 gut involvement. Optimism-corrected areas under the receiver-operator characteristic curve and Brier scores were 77.4 and 0.169 for 6-month mortality, respectively, and 80.0 and 0.169 for 12-month mortality. We identify risk factors associated with mortality and failure after second-line treatment of acute GVHD, provide historical benchmarks for assessment of FFS and OS in other studies, and propose predictive models for 6- and 12-month mortality that could be used to generate population-specific benchmarks.

Introduction

Acute graft-versus-host disease (GVHD) remains a significant cause of morbidity and mortality after allogenic hematopoietic cell transplantation (HCT). Initial treatment with corticosteroids produces sustained responses in less than 50% of patients. Many retrospective and prospective studies have evaluated interventions for patients with GVHD that has not responded to initial corticosteroid treatment, but very few randomized controlled trials have tested treatments for this indication. Evaluation of results in these studies has been complicated by the heterogeneity in the patient population (eg, patient age,
conditioning regimens, donor type, GVHD prophylaxis, type of HCT, and variable organ involvement), lack of a clear definition of steroid-refractory disease, and inconsistent study end points.

Despite decades of research and efforts to improve outcomes, patients who require second-line systemic treatment for acute GVHD have a poor prognosis. Review of experience at 2 different centers showed approximately 50% survival rates at 6 months and 30% to 35% at 12 months after starting second-line treatment. In randomized prospective trials, survival rates at 6 months after enrollment have ranged from 40% to 50% in studies that used horse antithymocyte globulin (ATG), rabbit ATG, or best available therapy as the control for investigational second-line treatment in adult or mixed adult and pediatric cohorts of patients. In a review of studies published between 1990 and 2011, the weighted average 6-month survival rate in retrospective or prospective studies of steroid-refractory or treatment-refractory acute GVHD was 49%, and in a subsequent review of studies published after 2011, the weighted average 6-month survival rate was 55%.

We conducted the current retrospective study to assess overall survival (OS) after second-line treatment of acute GVHD in patients who had received corticosteroids as initial treatment. We also evaluated failure-free survival (FFS), a composite end point defined as the earliest of death, relapse, or implementation of third-line treatment, and we examined potential risk factors for both mortality and failure. Last, we developed predictive models for both 6- and 12-month mortality and evaluated their performance by estimating the area under the receiver-operator characteristic curve (AUC) and Brier score for each outcome.

**Methods**

**Patients and data collection**

The study cohort included patients who received second-line systemic treatment of acute GVHD at the Fred Hutchinson Cancer Research Center (FHCRC)/Seattle Cancer Care Alliance from January 2006 through August 2018, regardless of the indication for transplantation, the conditioning regimen, graft source, donor relationship, or HLA matching. Patients had given consent allowing the use of medical records for research, as approved by the FHCRC Institutional Review Board. During this time, 3472 allogeneic transplantations were done at our center. Of these, 2161 received initial systemic treatment for acute GVHD. Nearly all were treated with prednisone at doses ranging from 0.5 to 2.0 mg/kg per day. The database identified 257 patients who received a new systemic treatment for acute GVHD after initial treatment with prednisone, representing 12% of all patients who received first-line treatment. Of these, we excluded 5 with recurrent malignancy or diagnosis of chronic GVHD before the change, 7 for whom the change was motivated by toxicity of an agent used for prophylaxis, 10 who had changes for other reasons such as low donor chimerism or difficulty controlling blood concentrations of agents used for prophylaxis, 1 who withdrew consent to use data for research, and 18 with inadequate information, leaving 216 patients included in the analysis. Follow-up clinical information was available from medical records submitted by referring physicians and from documentation generated by a dedicated long-term follow-up clinical program.

**Definitions**

Acute GVHD was graded according to previously described criteria. FFS was defined by the absence of treatment change, nonrelapse mortality, and recurrent malignancy. Abdominal pain requiring extended opioid treatment was defined as severe. Stage 4 GVHD was defined by the presence of overt hemorrhage or severe abdominal pain.

**Statistical analysis**

Both OS and FFS were treated as time-to-event outcomes. Failure time for the composite end point of FFS was defined as the earliest of death, relapse, or implementation of third-line treatment. Survival and FFS after second-line treatment were estimated using the Kaplan-Meier method. To estimate the contribution of each component of failure to FFS, cumulative incidence estimates of recurrent malignancy, nonrelapse mortality, and treatment change were derived, treating each event as a competing risk for the other.

Cox regression was used to assess the association of various factors with the hazards of failure associated with each of these outcomes as described in supplemental Methods. In Cox regression models, factors having a likelihood ratio \( P \leq 0.05 \) for association with mortality or failure in univariate testing were initially assessed for inclusion in appropriate multivariable models, but other factors were also considered even if the univariable model yielded a \( P > 0.05 \). Because of collinearity of various factors, the likelihood ratio test was used to determine which factors ultimately were included in the multivariable model for each end point. Factors at initiation of second-line treatment that were considered for inclusion in multivariable models and predictive models included pretransplant Hematopoietic Cell Transplant Comorbidity Index (HCT-CI), age at second-line treatment, time from transplant to first treatment, time from first treatment to second-line treatment, serum albumin and total bilirubin concentrations, any abdominal pain, presence of stage 4 gut involvement (defined as presence of severe abdominal pain requiring opioid treatment or presence of overt gastrointestinal bleeding), presence of fever, evidence of active infection, number of previous autologous or allogeneic transplants, skin GVHD stage at second-line treatment, percentage of body surface with rash, prednisone-equivalent steroid dose at time of second-line treatment, and agents used for second-line treatment. For the multivariable analyses, a model was fit from among variables other than the agent used for second-line treatment variables, and then the agent used for second-line treatment was added to this model. All noncategorical variables were modeled as continuous linear variables in the regression models.

Models for 6- and 12-month mortality were derived by using stepwise logistic regression (all patients had complete follow-up to 1 year), and the corresponding areas under the receiver-operator characteristic curves (AUC) and Brier scores were estimated as a means of assessing model discrimination and performance. The Brier score measures the accuracy of probability predictions and is equivalent to the mean square error but with outcomes being binary (dead [1] or alive [0]). The difference between the observed outcome (0 or 1) and the predicted probability of the outcome (ranging from 0 to 1) is squared for each patient, and the average of these squared differences is taken across all patients. As such, the score has a range from 0 to 1, where a score of 0 indicates perfect accuracy and a score of 1 indicates perfect inaccuracy. Because these...
performance measures were derived from the same data used to build the models, the inflation (for AUC) and deflation (for Brier score) were estimated using Harrell’s optimism.20,21 Harrell’s optimism for Brier scores and AUC was estimated from 200 bootstrap samples of 216 patients that were randomly generated, with replacement, and a model was generated for each bootstrap sample using stepwise logistic regression.22 This model was then applied to the original population of 216 patients, and the metrics from both the bootstrap sample and the original population were estimated. The average difference in metrics between these groups across the 200 bootstrap samples served as the estimate of optimism for each performance measure. These bootstrap samples serve as replicate development samples, allowing for correction of the overestimation of model performance that results from overfitting in the model-building process, thereby yielding an anticipated estimate of performance that might be expected in an independent data set.

Calibration for predictive models was assessed using calibration curves that show a locally estimated scatterplot smoother through the observed vs predicted probabilities of 6- and 12-month mortality based on the models. Calibration slope and intercept were

| Characteristic | n (%) |
|---------------|------|
| Median age of second-line treatment, y | 41 |
| Range | 1-76 |
| <12 y | 38 (18) |
| 12-17 | 17 (8) |
| 18-39 | 47 (22) |
| 40-59 | 70 (32) |
| ≥60 | 44 (20) |

| Characteristic | n (%) |
|---------------|------|
| Prednisone-equivalent steroid dose immediately before second-line treatment | |
| <1 mg/kg daily | 24 (11) |
| ≥1.0 but < 2.0 mg/kg daily | 64 (30) |
| ≥2.0 mg/kg daily | 128 (59) |
| Less than 14 d from transplant to first treatment | 45 (21) |
| Less than 14 d from first treatment to second treatment | 67 (31) |

| Bilirubin (mg/dL) at time of second-line treatment | |
| <1.0 | 124 (57) |
| 1.0-1.9 | 64 (30) |
| 2.0-3.9 | 16 (7) |
| ≥4.0 | 12 (6) |

| Albumin (g/dL) | |
| ≥4.0 | 15 (7) |
| 3.0-3.9 | 80 (37) |
| 2.0-2.9 | 92 (43) |
| 1.0-1.9 | 29 (13) |

| Presence of fever | |
| 10 (5) |
| 23 (11) |
| 61 (28) |
| 4 (2) |

| Presence of active infection | |
| 44 (20) |

*Low-risk diseases included chronic myeloid leukemia (CML) in chronic phase; high-risk diseases included acute leukemia not in remission, CML in blast crisis, refractory anemia with excess blasts (RAEB), or RAEB in transformation and myeloma; all other diseases and stages were categorized as standard risk.
estimated by regressing (via logistic regression) the observed 6- and 12-month probabilities on the logit of the predicted 6- and 12-month probabilities, respectively. The optimism in the slope and intercept was estimated using the same bootstrap approach described above.

Results

Patient characteristics

The analysis included 216 patients, representing 6.2% of the 3472 allogeneic transplantations at our center from January 2006 through August 2018. Patient characteristics are summarized in Table 1. The median age of patients was 41 years (range, 1-76 years). Of the 216 patients, 125 (58%) were prepared with high-dose conditioning regimens, 130 (60%) received mobilized PBSC graft, 33 (15%) had HLA-matched related donors, 100 (46%) had HLA-matched unrelated donors, and 83 (39%) had HLA-mismatched related or unrelated donors. One hundred seven (50%) patients had a pretransplant HCT-CI score of 3 or higher.

Patient characteristics at initial second-line treatment are summarized in Table 2. We could not assign overall GVHD grades at the onset of second-line treatment in this retrospective review because reliable stool volume measurements were not available for many patients. Immediately before initial second-line treatment, prednisone doses were <1.0 mg/kg daily in 24 patients (11%), ≥1.0 but <2.0 mg/kg daily in 64 (30%), and ≥2.0 mg/kg daily in 128 (50%). No patient received prednisone at ≥2.5 mg/kg per day. Sixty-seven (31%) started second-line treatment less than 14 days after the onset of systemic steroid treatment. At the onset of second-line treatment, 28 patients (13%) had serum total bilirubin concentrations ≥2.0 mg/dL, 29 (13%) had serum albumin concentrations <2.0 g/dL, 135 (63%) had diarrhea, 93 (43%) had abdominal pain, 9 (4%) had overt gastrointestinal bleeding, 98 (45%) had rash, 13 (6%) had fever, and 44 (20%) had documented concurrent active infection.

Outcomes after second-line treatments

Second-line treatments were ATG (21%), mycophenolate (20%), sirolimus (19%), α1 antitrypsin (9%), extracorporeal photopheresis (7%), infliximab (7%), or other (17%). Point estimates of OS after second-line treatment were 59% (95% confidence interval [CI], 52-65) and 52% (95% CI, 45-59) at 6 and 12 months, respectively (Figure 1A). Point estimates of FFS were 42% (95% CI, 35-48) and 37% (95% CI, 31-43) at 6 and 12 months, respectively (Figure 1B). New systemic treatment and death were the predominant categories of treatment failure. At 2 years, cumulative incidence frequencies for new systemic treatment and death were 29% and 31%, respectively, compared with 7% for recurrent malignancy (Figure 1C).

Risk factors associated with overall mortality

In univariate analyses (Table 3), factors statistically associated with risk of overall mortality included older patient age, longer interval time from transplant to first treatment, higher serum total bilirubin concentration (per mg/dL increase), lower serum albumin concentration (per g/dL decrease), presence of diarrhea, abdominal pain, stage 4 gut involvement, overt gastrointestinal bleeding or rash, higher steroid dose (per mg/kg prednisone equivalent), and higher HCT-CI. Given the association between abdominal pain and stage 4 gut involvement, these factors were combined into one as summarized in Table 3. Second-line treatment agent was also associated with risk of mortality in univariate analysis. Transplant number, transplant year, interval time from initial systemic treatment to onset of second-line treatment, fever, and active infection were not statistically associated with the risk of overall mortality. In multivariable
analysis (after forcing second-line treatment agent into the model), the risk factors for overall mortality that remained included older patient age, lower serum albumin concentration, higher serum total bilirubin concentration, and the combined stage 4 gut/abdominal pain. After adjusting for these factors, second-line treatment showed no statistically significant evidence of an association with the risk of mortality (global $P = .39$).

### Risk factors associated with treatment failure

In univariate analyses (Table 4), factors statistically associated with the risk of treatment failure included older patient age, longer interval time from initial systemic treatment to onset of second-line treatment, higher serum total bilirubin concentration (per mg/dL increase), lower serum albumin concentration (per g/dL decrease), presence of diarrhea, abdominal pain, stage 4 gut involvement, abdominal pain/stage 4 gut involvement, overt gastrointestinal bleeding or rash, higher steroid dose (per mg/kg prednisone equivalent), and higher HCT-CI. Second-line treatment agent was also associated with risk of failure in univariate analysis. Transplant number, transplant year, interval time from transplant to initial systemic treatment, fever, and active infection were not statistically associated with risk of treatment failure. In multivariable analysis, lower serum albumin concentration, higher total serum bilirubin concentration, and the combined variable stage 4 gut/abdominal pain showed an association with treatment failure. After adjusting for these factors, second-line treatment agent showed no statistically significant evidence of an association with the risk of failure (global $P = .69$).

### Predictive models for 6- and 12-month mortality

The same factors identified in the Cox model for overall mortality were identified for predictive models for both 6- and 12-month mortality. Summarized in Table 5 are various metrics for both discrimination and calibration for these models, along with corresponding optimism-corrected estimates. Figure 2 shows the calibration curves for 6- and 12-month mortality. Also shown in Table 5 are the estimated coefficients for the various factors included in the models.

### Table 3. Patient, disease, and treatment characteristics associated with risk of mortality

| Characteristic at start of second-line treatment | Univariate analysis | Multivariable analysis |
|-----------------------------------------------|---------------------|-----------------------|
|                                               | HR                  | 95% CI                | $P$       | HR                  | 95% CI                | $P$       |
| Age at second-line treatment (per decade)     | 1.34                | 1.22-1.46             | <.0001    | 1.22                | 1.10-1.35             | .0002     |
| Weeks from transplant to first treatment (per week) | 1.09                | 1.02-1.16             | .007      |                    |                       |           |
| Bilirubin (per mg/dL)                        | 1.16                | 1.07-1.26             | .0004     | 1.17                | 1.07-1.28             | .001      |
| Albumin (per g/dL decrease)                  | 2.53                | 1.96-3.25             | <.0001    | 1.83                | 1.30-2.57             | .0005     |
| Diarrhea                                     | 2.06                | 1.42-3.00             | .0002     |                    |                       |           |
| Any abdominal pain                           | 2.54                | 1.80-3.58             | <.0001    |                    |                       |           |
| Stage 4 gut                                  | 3.57                | 2.42-5.28             | <.0001    |                    |                       |           |
| No abdominal pain, no stage 4 gut            | Ref†                | NA                    | NA        | Ref†                | NA                    | NA        |
| Any abdominal pain, no stage 4 gut*          | 1.81                | 1.20-2.75             | .005      | 1.17                | 0.71-1.91             | .54       |
| Any abdominal pain with stage 4 gut          | 4.39                | 2.86-6.73             | <.0001    | 2.96                | 1.76-4.98             | <.0001    |
| Overt gastrointestinal hemorrhage            | 4.07                | 2.04-8.13             | <.0001    |                    |                       |           |
| HCT-CI (per unit)                            | 1.24                | 1.14-1.34             | <.0001    |                    |                       |           |
| Steroid dose (per mg/kg prednisone equivalent) | 1.74                | 1.22-2.47             | .002      |                    |                       |           |
| Skin stage (per stage)                       | 0.83                | 0.73-0.94             | .004      |                    |                       |           |
| Body surface with rash (per 10%)             | 0.92                | 0.87-0.97             | .001      |                    |                       |           |
| Number of prior transplants                  | 1.04                | 0.70-1.55             | .84       |                    |                       |           |
| Transplant year (per year)                   | 1.04                | 0.99-1.09             | .09       |                    |                       |           |
| Weeks from first treatment to second treatment (per week) | 0.97                | 0.93-1.01             | .16       |                    |                       |           |
| Fever                                        | 0.64                | 0.26-1.57             | .33       |                    |                       |           |
| Active infection                             | 0.81                | 0.51-1.26             | .35       |                    |                       |           |
| Agent                                        |                     |                       |           |                     |                       |           |
| Extracorporeal photopheresis                 | Ref†                | NA                    | NA        | Ref†                | NA                    | NA        |
| α-1 antitrypsin                              | 3.72                | 1.34-10.35            | .01       | 1.45                | 0.51-4.14             | .49       |
| ATG                                          | 4.42                | 1.73-11.25            | .002      | 1.74                | 0.68-4.58             | .27       |
| Infliximab                                    | 2.26                | 0.76-6.75             | .14       | 1.33                | 0.43-4.09             | .62       |
| Mycophenolate mofetil (not used for prophylaxis) | 1.61                | 0.61-4.23             | .34       | 1.69                | 0.62-4.64             | .31       |
| Sirolimus                                    | 1.40                | 0.52-3.79             | .50       | 1.47                | 0.54-3.98             | .45       |
| Other                                        | 3.37                | 1.29-8.75             | .01       | 2.51                | 0.95-6.59             | .08       |

HR, hazard ratio; NA, not applicable.

*This group also included the 2 patients with no abdominal pain and stage 4 gut involvement because of overt hemorrhage.

†Reference groups were used as the comparison for other groups.
The values for these coefficients can be used to estimate the predicted probability of 6- or 12-month death as shown for a hypothetical patient in Figure 2.

Discussion

This retrospective study was designed to examine outcomes and risk factors associated with treatment failure and overall mortality after second-line treatment for acute GVHD patients at our Center. The incidence of second-line treatment is lower than we had expected and is lower than the 36% incidence reported recently by Axt et al.16 We surmise that many other patients had GVHD that met criteria for steroid-refractory GVHD23 but did not receive second-line treatment because of (1) confidence that a temporary increase in steroid dose and a slower subsequent steroid taper with or without topical treatment could control the disease, especially in the context of an initial flare, (2) lack of confidence in the efficacy of second-line agents, or (3) concern about the side effects of second-line agents. The distribution of reasons for not using a second-line systemic agent could not be determined in this retrospective study. Small numbers of patients had prednisone-equivalent doses <0.5 mg/kg per day at the onset of second-line treatment. We surmise that decisions to use a second-line systemic agent for these patients were motivated by steroid side effects or repeated inability to taper steroid doses. To the best of our knowledge, other information to determine the extent to which practices at our center differ from those at other centers is not available.

We observed a high degree of collinearity in certain disease-related risk factors, and due, at least in part to this collinearity, the list of risk factors in univariate analyses foreshortened to only 3 risk factors for treatment failure (serum albumin, total bilirubin concentration, and abdominal pain/stage-4 gut involvement), and 4 risk factors for mortality (patient age, serum albumin and total bilirubin concentrations, and abdominal pain/stage 4 gut involvement) in the stepwise multivariable logistic regression analyses. These same 4 factors contributed to models for both 6- and 12-month mortality, for which we

Table 4. Patient, disease, and treatment characteristics associated with risk of failure (new treatment, relapse, or death)

| Characteristic at start of second-line treatment | Univariate analysis | Multivariable analysis |
|--------------------------------------------------|---------------------|-----------------------|
|                                                  | HR                  | 95% CI                | P        | HR                  | 95% CI                | P        |
| Age at second-line treatment (per decade)        | 1.16                | 1.07-1.25             | .0002    | 1.18                | 1.08-1.28             | .0002    |
| Weeks from transplant to first treatment (per week) | 1.05                | 0.99-1.12             | .10      | 1.05                | 0.99-1.12             | .10      |
| Bilirubin (per mg/dL increase)                   | 1.15                | 1.07-1.24             | .0002    | 1.18                | 1.08-1.28             | .0002    |
| Albumin (per g/dL decrease)                      | 2.27                | 1.82-2.84             | <.0001   | 1.87                | 1.42-2.46             | <.0001   |
| Diarrhea                                         | 2.13                | 1.50-3.01             | <.0001   | 1.87                | 1.42-2.46             | <.0001   |
| Any abdominal pain                               | 2.10                | 1.53-2.89             | <.0001   | 1.39                | 0.94-2.06             | .10      |
| Stage 4 gut                                      | 2.61                | 1.81-3.78             | <.0001   | 2.61                | 1.81-3.78             | <.0001   |
| No abdominal pain, no stage 4 gut                | Ref†                | NA                    | NA       | Ref†                | NA                    | NA       |
| Any abdominal pain, no stage 4 gut*              | 1.65                | 1.12-2.41             | .01      | 1.17                | 0.71-1.91             | .54      |
| Any Abdominal pain with stage 4 gut              | 3.06                | 2.05-4.56             | <.0001   | 2.96                | 1.76-4.98             | <.0001   |
| Overt GI hemorrhage                              | 3.53                | 1.78-7.01             | .0003    | 3.53                | 1.78-7.01             | .0003    |
| HCT-CI (per unit)                                | 1.12                | 1.04-1.22             | .004     | 1.12                | 1.04-1.22             | .004     |
| Steroid dose (per mg/kg prednisone equivalent)   | 1.73                | 1.25-2.39             | <.0001   | 1.73                | 1.25-2.39             | <.0001   |
| Skin stage (per stage)                           | 0.78                | 0.69-0.88             | <.0001   | 0.78                | 0.69-0.88             | <.0001   |
| Body surface with rash (per 10%)                 | 0.90                | 0.86-0.95             | <.0001   | 0.90                | 0.86-0.95             | <.0001   |
| Number of prior transplants                      | 1.19                | 0.84-1.70             | .33      | 1.19                | 0.84-1.70             | .33      |
| Transplant year (per year)                       | 1.03                | 0.99-1.08             | .15      | 1.03                | 0.99-1.08             | .15      |
| Weeks from first treatment to second treatment (per week) | 0.96                | 0.92-1.00             | .05      | 0.96                | 0.92-1.00             | .05      |
| Fever                                            | 0.82                | 0.59-1.76             | .62      | 0.82                | 0.59-1.76             | .62      |
| Active infection                                 | 0.88                | 0.58-1.32             | .54      | 0.88                | 0.58-1.32             | .54      |
| Agent                                            |                      |                       |          |                      |                       |          |
| Extracorporeal photopheresis                     | Ref†                | NA                    | NA       | Ref†                | NA                    | NA       |
| α-1 antitrypsin                                  | 3.08                | 1.37-6.94             | .007     | 1.58                | 0.68-3.67             | .29      |
| ATG                                              | 2.07                | 1.00-4.28             | .05      | 1.12                | 0.52-2.40             | .77      |
| Infliximab                                       | 1.69                | 0.71-4.01             | .24      | 1.04                | 0.43-2.52             | .94      |
| Mycophenolate mofetil (not used for prophylaxis) | 0.88                | 0.41-1.88             | .73      | 1.03                | 0.47-2.27             | .94      |
| Sirolimus                                        | 0.96                | 0.45-2.08             | .92      | 0.96                | 0.45-2.11             | .96      |
| Other                                            | 1.59                | 0.75-3.37             | .23      | 1.42                | 0.67-3.04             | .36      |

HR, hazard ratio; NA, not applicable.

*This group also included the 2 patients with no abdominal pain and stage 4 gut involvement because of overt hemorrhage.

†Reference groups were used as the comparison for other groups.
supply a formula to predict the patient-specific probability of each outcome based on values for each factor.

Higher total serum bilirubin concentration and lower serum albumin concentration were identified as risk factors associated with both FFS and OS. Previous studies have shown that high serum total bilirubin concentrations predict a high risk of mortality in patients with acute GVHD and more generally among all patients who underwent HCT. In patients with GVHD, hyperbilirubinemia has been attributed to apoptotic bile duct epithelial damage or destruction, endothelial inflammation, pericholangitis, and cytokine effects. Low serum albumin concentration because of gastrointestinal protein loss has been shown as an early, often preclinical phenomenon in patients with acute Gl GVHD after HCT and is associated with poor survival. Protein-losing enteropathy likely occurs through leaky epithelial tight junctions in the midgut epithelium, allowing transit of protein from the lamina propria into the lumen and reverse translocation of luminal bacteria and endotoxin into the lamina propria and thence into the circulation. Our finding that hypoalbuminemia is associated with an increased risk of mortality corresponds with previous results showing that grade 3 to 4 severity at the onset of second-line treatment is a risk factor for mortality in patients with steroid-refractory or -dependent acute GVHD. Other biomarkers such as fecal calprotectin, fecal α1-antitrypsin, and plasma citrulline have also been identified as useful indicators of protein-losing enteropathy and poor prognosis in patients with gastrointestinal GVHD. In addition, high serum concentrations of regenerat-

Table 5. Performance metrics from predictive models and estimated coefficients from the models

| Parameter                           | 6-mo mortality | 12-mo mortality |
|-------------------------------------|----------------|-----------------|
| Number of events                    | 88             | 103             |
| **Performance metrics**             |                |                 |
| AUC                                 | 85.0           | 85.9            |
| Optimism-corrected AUC              | 77.4           | 80.0            |
| Brier score                         | 0.153          | 0.152           |
| Optimism-corrected Brier score      | 0.169          | 0.169           |
| Calibration slope                   | 1.0000         | 1.0003          |
| Calibration intercept               | −0.00043       | 0.00141         |
| Optimism-corrected intercept        | 0.85           | 0.85            |
| Optimism-corrected intercept        | −0.09          | −0.10           |
| **Intercept and coefficients**      |                |                 |
| Intercept                           | 1.1149         | 1.4284          |
| Patient age                         | 0.0286         | 0.0331          |
| Bilirubin                           | 0.2520         | 0.2777          |
| Albumin                             | −1.2006        | −1.2698         |
| Abdominal pain, no stage 4 gut      | −0.0661        | 0.3550          |
| Abdominal pain with stage 4 gut     | 1.8603         | 1.7278          |

The estimated 59% 6-month survival rate in the current study may be higher than expected when compared with most previous studies, possibly because of a high proportion of pediatric patients, the exclusion of patients who had previously received agents other than corticosteroids for treatment of acute GVHD and improvements in prevention of infection. The high survival rate makes it more difficult to demonstrate improved survival even with a highly effective investigational treatment. Improved OS might not stand as a bar of success for current studies at this early stage, but the field should hold it as a goal to reach in the future.
Limitations of our study include the retrospective design and the small numbers of patients that impaired our ability to observe demonstrable differences in survival among the treatment groups. We could not evaluate GVHD grade, because accurate stool volume measurements were not available, and we did not attempt to assess response in this retrospective study. Despite these limitations, the results of this study are important because they provide historical benchmarks for the longer-term outcomes of OS and FFS that would be useful in assessing results of future single-arm and controlled trials testing new agents for second-line treatment of acute GVHD. Our identified risk factors associated with mortality and treatment failure will also help evaluate whether differences between trials or study arms might be attributable to patient selection rather than the effects of the investigational product. These same risk factors may provide valuable prognostic information in counseling patients who require second-line treatment for acute GVHD. The models for 6- and 12-month mortality could be useful in this regard, as well as in calculating an appropriate study-specific benchmark from a given study based on the patient demographics for the 4 factors identified. Although we used Harrell’s optimism as a means of internal validation, these models should be further assessed in external independent cohorts such as REACH-2 study and their performance estimated as noted above.

Previous studies have shown that a biomarker combination of Reg3α and ST2 with or without tumor necrosis factor receptor 1 can be used to predict dichotomized low and high risks of nonrelapse mortality from the onset of acute GVHD and from day 7 afterward.

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**Figure 2.** Statistical models predict the probability of mortality at 6 and 12 months after second-line treatment. Calibration curves for mortality at (A) 6 and (B) 12 months after starting second-line treatment with (C) an example for a hypothetical individual patient. The solid blue line shows the fit between the observed probabilities of mortality (y axis) with the estimated probabilities of mortality as predicted by the model (x axis) (see Methods for details). The fit is obtained by using locally estimated scatterplot smoothing. This approach is like standard linear least-squares regression, but this simpler model is fit to localized subsets of the data, leading to a more flexible representation of the fit between the predicted and observed outcomes than could be achieved assuming a linear association across the entire span of the data. The dashed red line shows results that would be expected for a perfect correlation. The shaded area shows the pointwise 95% CIs of the observed probabilities of mortality across the range of predicted probabilities. In the example, values for each risk factor are multiplied by the respective coefficients. The sum of the products plus the intercept is used to predict the probability of mortality from the formula in the bottom row. In this example, the predicted probability of mortality is 0.39 at 6 months and 0.58 at 12 months.
regardless of second-line treatment.\textsuperscript{38,42,43} This biomarker combination has not yet been tested for prediction of nonrelapse mortality or survival after the onset of second-line treatment, and calibration to provide survival predictions for individual patients with the use of this biomarker panel has not been developed. In future studies, it would be of considerable interest to compare the accuracy and utility of the biomarker-based approach vs the clinical risk factor approach and to evaluate a combination of the 2 approaches for calibrated prediction of survival after second-line systemic treatment for acute GVHD.

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Authorship

Contribution: P.V. collected data and drafted the manuscript; P.A.C. and M.L.S. collected data; T.A.G. analyzed data; and P.J.M. collected data and drafted the manuscript. All authors critically revised the manuscript and reviewed and approved the final manuscript.

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References

1. Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. \textit{Biol Blood Marrow Transplant.} 2012;18(8):1150-1163.

2. Van Lint MT, Milone G, Leotta S, et al. Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day +5 responders and no advantage for nonresponders receiving anti-thymocyte globulin. \textit{Blood.} 2006;107(10):4177-4181.

3. Macmillan ML, Couriel D, Weisdorf DJ, et al. A phase 2/3 multicenter randomized clinical trial of ABX-CBL versus ATG as secondary therapy for steroid-resistant acute graft-versus-host disease. \textit{Blood.} 2007;109(6):2657-2662.

4. Muroi K, Miyamura K, Okada M, et al. Bone marrow-derived mesenchymal stem cells (JR-031) for steroid-refractory grade III or IV acute graft-versus-host disease: a phase II/III study. \textit{Int J Hematol.} 2016;103(2):243-250.

5. Socié G, Vigouroux S, Yakoub-Agha I, et al. A phase 3 randomized trial comparing inolimomab vs usual care in steroid-resistant acute GVHD. \textit{Blood.} 2017;129(5):643-649.

6. Kebridea P, Hayes J, Daly A, et al. A phase 3 randomized study of remestemcel-H versus placebo added to second-line therapy in patients with steroid-refractory acute graft-versus-host disease. \textit{Biol Blood Marrow Transplant.} 2020;26(5):835-844.

7. Zeiser R, von Bubnoff N, Butler J, et al; REACH2 Trial Group. Ruxolitinib for glucocorticoid-refractory acute graft-versus-host disease. \textit{N Engl J Med.} 2020;382(19):1800-1810.

8. Martin PJ. How I treat steroid-refractory acute graft-versus-host disease. \textit{Blood.} 2020;135(19):1630-1638.

9. Malard F, Huang XJ, Sim JPY. Treatment and unmet needs in steroid-refractory acute graft-versus-host disease. \textit{Leukemia.} 2020;34(5):1229-1240.

10. Martin PJ, Inamoto Y, Flowers ME, Carpenter PA. Secondary treatment of acute graft-versus-host disease: a critical review. \textit{Biol Blood Marrow Transplant.} 2012;18(7):982-988.

11. Deeg HJ. How I treat refractory acute GVHD. \textit{Blood.} 2007;109(10):4119-4126.

12. McDonald GB. How I treat acute graft-versus-host disease of the gastrointestinal tract and the liver. \textit{Blood.} 2016;127(12):1544-1550.

13. Toubai T, Magenau J. Immunopathology and biology-based treatment of steroid-refractory graft-versus-host disease. \textit{Blood.} 2020;136(4):429-440.

14. Zeiser R, Blazar BR. Acute graft-versus-host disease: biologic process, prevention, and therapy. \textit{New Engl J Med.} 2017;377(22):2167-2179.

15. Xhaard A, Rocha V, Bueno B, et al. Steroid-refractory acute GVHD: lack of long-term improved survival using new generation anticytokine treatment. \textit{Biol Blood Marrow Transplant.} 2012;18(3):406-413.

16. Ast L, Naumann A, Toennis J, et al. Retrospective single center analysis of outcome, risk factors and therapy in steroid refractory graft-versus-host disease after allogeneic hematopoietic cell transplantation. \textit{Bone Marrow Transplant.} 2019;54(11):1805-1814.

17. Socié G, Milpied N, Yakoub-Agha I, et al. Long-term follow-up of a phase 3 clinical trial of inolimomab for the treatment of primary steroid refractory aGVHD. \textit{Blood Adv.} 2019;3(2):184-186.

18. Leisenring WM, Martin PJ, Petersdorf EW, et al. An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens. \textit{Blood.} 2006;108(2):749-755.
19. Gooley TALW, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18(6):695-706.

20. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15(4):361-387.

21. Miao Y, Cenzer IS, Boscardin WJ. Estimating Harrell’s optimism on predictive indices using bootstrap samples. SAS Global Forum. 2013; https://support.sas.com/resources/papers/proceedings13/504-2013.pdf.

22. Harrell FE Jr, Lee KL, Califf RM, Pryor DB, Rosati RA. Regression modelling strategies for improved prognostic prediction. Stat Med. 1984;3(2):143-152.

23. Przepiorka D, Luo L, Subramaniam S, et al. FDA approval summary: ruxolitinib for treatment of steroid-refractory acute graft-versus-host disease. Oncologist. 2020;25(2):e328-e334.

24. Castilla-Lorente C, Martin PJ, McDonald GB, et al. Prognostic factors and outcomes of severe gastrointestinal GVHD after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2014;49(7):966-971.

25. Gooley TA, Rajvanshi P, Schoch HG, McDonald GB. Serum bilirubin levels and mortality after myeloablative allogeneic hematopoietic cell transplantation. Hepatology. 2005;41(2):345-352.

26. MacMillan ML, Robin M, Harris AC, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. Biol Blood Marrow Transplant. 2015;21(4):761-787.

27. Robin M, Porcher R, de Castro R, et al. Initial liver involvement in acute GVHD is predictive for nonrelapse mortality. Transplantation. 2009;88(9):1131-1136.

28. Rezvani AR, Storer BE, Storb RF, et al. Decreased serum albumin as a biomarker for severe acute graft-versus-host disease after reduced-intensity allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2011;17(1):1594-1601.

29. Ayuk F, Bussmann L, Zabelina T, et al. Serum albumin level predicts survival of patients with gastrointestinal acute graft-versus-host disease after allogeneic stem cell transplantation. Ann Hematol. 2014;93(5):855-861.

30. van der Velden WJ, Herbers AH, Brüggemann RJ, Feuth T, Peter Donnelly J, Blijlevens NM. Citrulline and albumin as biomarkers for gastrointestinal mucositis in recipients of hematopoietic SCT. Bone Marrow Transplant. 2013;48(7):977-981.

31. Broglie L, Rademaker A, Galvin J, et al. Fecal calprotectin and serum albumin as markers of gastrointestinal graft versus host disease. Hematol Oncol Stem Cell Ther. 2018;11(3):169-174.

32. Andrews C, McLean MH, Durum SK. Cytokine tuning of intestinal epithelial function. Front Immunol. 2018;9:1270.

33. Al-Sadi R, Storer BE, Storb RF, et al. Decreased serum albumin as a biomarker for severe acute graft-versus-host disease after reduced-intensity allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2011;17(1):1594-1601.

34. Weisdorf SA, Salati LM, Longsdorf JA, Ramsay NK, Sharp HL. Graft-versus-host disease of the intestine: a protein losing enteropathy characterized by fecal alpha 1-antitrypsin. Gastroenterology. 1983;85(5):1076-1081.

35. Rodriguez-Otero P, Porcher R, Peffault de Latour R, et al. Fecal calprotectin and alpha-1 antitrypsin predict severity and response to corticosteroids in gastrointestinal graft-versus-host disease. Blood. 2012;119(12):2960-2963.

36. Levine JE, Huber E, Hammer ST, et al. Low Paneth cell numbers at onset of gastrointestinal graft-versus-host disease identify patients at high risk for nonrelapse mortality. Blood. 2013;122(8):1505-1509.

37. Major-Monfried H, Renteria AS, Pawarode A, et al. MAGIC biomarkers predict long-term outcomes for steroid-resistant acute GVHD. Blood. 2018;131(25):2846-2855.

38. Jaga M, Perales MA, Schroeder MA, et al. Ruxolitinib for the treatment of steroid-refractory acute GVHD (REACH1): a multicenter, open-label phase 2 trial. Blood. 2020;135(20):1739-1749.

39. Van Calster B, Nieboer D, Vergouwe Y, De Cock B, Pencina MJ, Steyerberg EW. A calibration hierarchy for risk models was defined: from utopia to empirical data. J Clin Epidemiol. 2016;74:167-176.

40. Ewout W, Steyerberg SEB, Moll HA, Grobbée DE, Moons KGM. Internal and external validation of predictive models: a simulation study of bias and precision in small samples. J Clin Epidemiol. 2003;56(5):441-447.

41. Levine JE, Braun TM, Harris AC, et al; Blood and Marrow Transplant Clinical Trials Network. A prognostic score for acute graft-versus-host disease based on biomarkers: a multicentre study. Lancet Haematol. 2015;2(1):e21-e29.

42. Hartwell MJ, Özbek U, Holler E, et al. An early-biomarker algorithm predicts lethal graft-versus-host disease and survival. JCI Insight. 2017;2(3):e89798.