SUPPLEMENTARY APPENDIX

Response endpoints and failure-free survival after initial treatment for acute graft-*versus*-host disease

Yoshihiro Inamoto,[1] Paul J. Martin,[1,2] Barry E. Storer,[1,3] Marco Mielcarek,[1,2] Rainer F. Storb,[1,2] and Paul A. Carpenter[1,4]

[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle; [2]Department of Medicine, University of Washington School of Medicine, Seattle; [3]Department of Biostatistics, University of Washington School of Medicine, Seattle; and [4]Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA

 doi:10.3324/haematol.2013.093062
Manuscript received on June 11, 2013. Manuscript accepted on September 13, 2013.
Correspondence: pcarpent@fhcrc.org.

Online Supplement

Methods

Patients

The study cohort included 303 relapse-free adult patients who received initial systemic steroid treatment for grades IIb-IV acute GVHD after a first allogeneic bone marrow or mobilized blood cell transplantation at the Fred Hutchinson Cancer Research Center / Seattle Cancer Care Alliance between January 2000 and December 2005. Patients with grade IIa GVHD were excluded, since they received less uniform treatment, generally have a very good prognosis, and therefore may not be a candidate for trials using high-dose glucocorticoids.[1, 2] All patients signed consent forms allowing the use of medical records for research related to outcomes after transplantation, and the institutional review board of the Fred Hutchinson Cancer Research Center approved the study.

Definitions

Acute GVHD was prospectively diagnosed, staged and graded according to the established criteria.[3, 4] Grade IIa GVHD was defined as stage 1 gastrointestinal (GI) involvement with stage 0-2 skin involvement and no liver involvement. Grade IIb GVHD was defined as stage 3 skin involvement or stage 1 liver involvement with or without stage 1 GI involvement.[5] The intensity of conditioning regimens was defined as high-intensity or reduced-intensity as described elsewhere.[5, 6] CR was defined as the complete resolution of acute GVHD manifestations in all organs. Traditional PR was defined as improvement in GVHD stage in at least one of the initially involved organs without complete resolution and without worsening in any other organs. VGPR was retrospectively defined through detailed chart review when patients otherwise met the CR criteria but had at least one of the following manifestations (see Supplementary Table S1 for additional details): (1) non-progressive stage 1 rash, not counting residual faint erythema or hyperpigmentation; (2) resolving elevations of total serum bilirubin concentration <25% of

baseline; (3) minimal GI symptoms as defined by tolerance of oral intake, predominantly formed stools, no abdominal cramping, and no more than occasional nausea or vomiting.[7] Other PR was defined as any traditional PR that did not meet criteria for VGPR. No response (NR) was defined as the same stage of GVHD in all organs or progression of GVHD in any organ. Chronic GVHD was diagnosed according to the National Institutes of Health consensus criteria.[8]

Failure-free survival (FFS) was defined by the absence of 3 types of treatment failure: second-line systemic treatment for acute GVHD, nonrelapse mortality (NRM) and recurrent malignancy during initial treatment. Onset of chronic GVHD was considered as a competing risk for all 3 types of failure. Second-line treatment was defined as any additional systemic treatment not used for initial treatment of acute GVHD. Consistent with the BMT CTN 0302 and 0802 studies,[9] second-line treatment also included an increase in the prednisone-equivalent steroid dose to ≥2.5 mg/kg/day because of flare during the steroid taper. Recurrent malignancy was defined as hematologic relapse or any unplanned intervention intended to prevent progression of malignancy in patients with molecular, cytogenetic, flow cytometric or any other evidence of malignant disease after transplantation.

Prophylaxis and treatment of GVHD

GVHD prophylaxis included a calcineurin inhibitor with either methotrexate or mycophenolate mofetil (MMF) after high-intensity conditioning, and a calcineurin inhibitor and MMF after reduced-intensity conditioning.[5] Prednisone or methylprednisolone was used for initial treatment of acute GVHD. The initial prednisone-equivalent dose was 2 mg/kg/day in majority of patients according to the institutional standard practice and the initial dose was 1 mg/kg/day for some patients at the attending's discretion.[5] Daily prednisone doses >1.0 mg/kg/day were given in divided doses twice daily, while lower doses were given once daily in the morning. After improvement of GVHD manifestations, steroid doses were tapered over 5 to 8 weeks according

to the institutional standard practice. Decisions to initiate second-line systemic treatment were made at the discretion of the attending physician.

Statistical analysis

Cumulative incidence estimates of treatment failure defined as the first event of recurrent malignancy, NRM or systemic treatment change during initial treatment were derived, treating each event as a competing risk for the other two.[10] Onset of chronic GVHD during initial treatment was treated as a competing risk for all 3 types of failure. Cumulative incidence estimates of NRM were also derived, treating recurrent malignancy as the only competing risk.

Cox regression models were used to identify risk factors for treatment failure. Logistic regression models were used to identify factors associated with day 28 response. Covariates included were patient age at transplantation (per decade), patient sex, donor-patient gender combination, disease risk, graft source, HLA matching, donor relation, intensity of conditioning regimen, GVHD prophylaxis, GVHD grade at initial treatment, organ involvement at initial treatment (any skin vs. no skin, any liver vs. no liver, any GI tract vs. no GI, or skin only vs. others), time from transplantation to initial treatment for acute GVHD (>20 days vs. ≤20 days) and initial dose of steroids. Factors having a likelihood ratio *P*-value ≤.05 for association with failure in univariate testing were included in a multivariate model. A backward elimination procedure was used to exclude risk factors until the *P*-value of the likelihood ratio test for all remaining risk factors was ≤.05.

Sensitivity and specificity analyses were used to evaluate each response definition in predicting the absence of treatment failure at 6 months after initial treatment. Positive and negative predictive values were also calculated. As in previous studies,[11, 12] failures before response assessment were included as a NR category in these analyses in order to correspond to the conduct and interpretation of clinical trials. The 6-month time point for assessing longer-term outcomes was used for consistency with previous studies.[11, 13] Plots of sensitivity and

specificity were examined to evaluate the merits of incorporating a minimum percent reduction of the initial steroid dose in the response definition.

Interpretation of sensitivity and specificity

Sensitivity denotes the proportion of short-term responders among patients without longer-term treatment failure. Low sensitivity indicates a high incidence of false-negative results (type 2 error). Specificity denotes the proportion of short-term non-responders among patients with longer-term treatment failure. Low specificity indicates a high incidence of false-positive results (type 1 error). Clinical trials have less tolerance for false-positive results than for false-negative results. Therefore, specificity was prioritized over sensitivity when sensitivity and specificity showed a balanced trade-off.

Supplementary Table S1. Detailed definition of VGPR

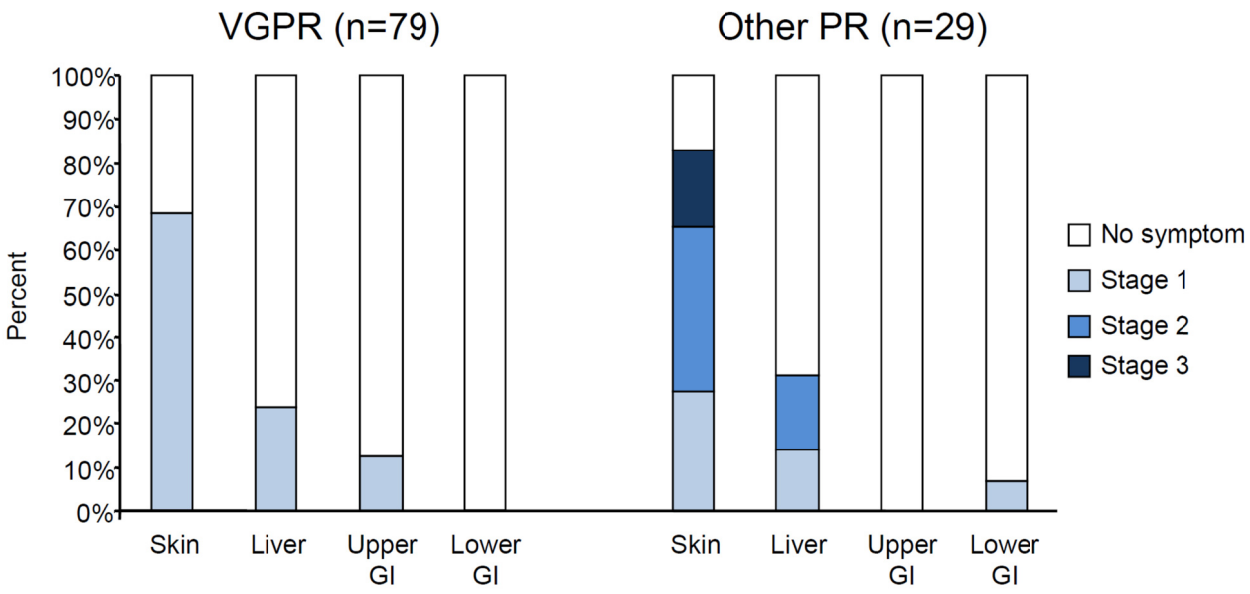
Skin	The terminology allows active erythematous rash involving <25% of the body surface area. Rash that is pink, fading, or turning to brown is not included in the measurement, because these findings indicate resolving lesions.
Liver	The terminology allows for persisting low level hyperbilirubinemia that might be related to antecedent regimen-related hepatotoxicity, concomitant hemolysis, or administration of hepatotoxic agents such as voriconazole, cyclosporine, or total parental nutrition (TPN), or other factors such as sepsis. A serum total bilirubin concentration of <2 mg/dL approximates normal values, and a reduction to <25% of the baseline concentration provides strong evidence of progression toward normal liver function among patients with levels ≥2 mg/dL.
Gut	Criteria in the terminology were selected to indicate that gut function and water resorption in the colon are approaching normal. These criteria have some imprecision and rely heavily on patient recall, rather than measurement of stool volume, but they can be easily used for outpatients. In certain cases, it might be necessary to make allowances for the effects of pretransplant diseases that cause diarrhea.

Cited from Biol Blood Marrow Transplant. 2009;15(7):777-784.[7]

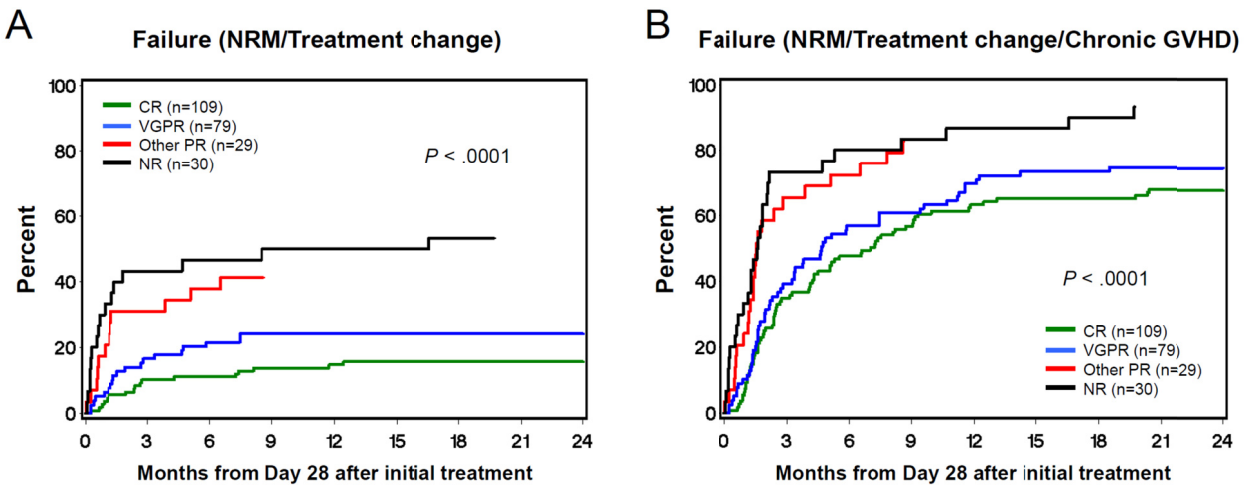
Supplementary Table S2. Multivariate analysis of factors associated with CR/VGPR at day 28

Factor	N	Odds ratio (95% CI)	*P*
HLA and donor type			
HLA-matched related donor	80	1.00 (reference)	
HLA-matched unrelated donor	138	0.33 (0.17-0.63)	.0008
HLA-mismatched donor	85	0.32 (0.16-0.64)	.001
Organ involvement at initial treatment			
Skin only	93	1.00 (reference)	
Other organs	210	0.39 (0.23-0.69)	.001

Supplementary Figure S1. Comparison of symptom burden between VGPR and Other PR at day 28.



Supplementary Figure S2. Cumulative incidence of treatment failure according to day 28 response. (A) Treatment failure included nonrelapse mortality (NRM) and treatment change. (B) Treatment failure included NRM, treatment change and chronic GVHD.



References

1. Weisdorf DJ, Snover DC, Haake R, Miller WJ, McGlave PB, Blazar B, et al. Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. Blood. 1990;76(3):624-9.
2. Hockenbery DM, Cruickshank S, Rodell TC, Gooley T, Schuening F, Rowley S, et al. A randomized, placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease. Blood. 2007;109(10):4557-63.
3. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-8.
4. Leisenring WM, Martin PJ, Petersdorf EW, Regan AE, Aboulhosn N, Stern JM, et al. An acute graft-versus-host disease activity index to predict survival after hematopoietic cell transplantation with myeloablative conditioning regimens. Blood. 2006;108(2):749-55.
5. Mielcarek M, Storer BE, Boeckh M, Carpenter PA, McDonald GB, Deeg HJ, et al. Initial therapy of acute graft-versus-host disease with low-dose prednisone does not compromise patient outcomes. Blood. 2009;113(13):2888-94.
6. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-33.
7. Martin PJ, Bachier CR, Klingemann HG, McCarthy PL, Szabolcs P, Uberti JP, et al. Endpoints for clinical trials testing treatment of acute graft-versus-host disease: a joint statement. Biol Blood Marrow Transplant. 2009;15(7):777-84.
8. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic

graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945-56.

9. Alousi AM, Weisdorf DJ, Logan BR, Bolanos-Meade J, Carter S, Difronzo N, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. Blood. 2009;114(3):511-7.

10. Gooley TA, 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.

11. Levine JE, Logan B, Wu J, Alousi AM, Ho V, Bolanos-Meade J, et al. Graft-versus-host disease treatment: predictors of survival. Biol Blood Marrow Transplant. 2010;16(12):1693-9.

12. MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. Blood. 2010;115(26):5412-7.

13. Saliba RM, Couriel DR, Giralt S, Rondon G, Okoroji GJ, Rashid A, et al. Prognostic value of response after upfront therapy for acute GVHD. Bone Marrow Transplant. 2012;47(1):125-31.