Evaluation of pentostatin in corticosteroid-refractory chronic graft-versus-host disease in children: a Pediatric Blood and Marrow Transplant Consortium study

David A. Jacobsohn¹, Andrew L. Gilman², Alfred Rademaker¹, Brittan Browning³, Michael Grimley⁴, Leslie Lehmann⁵, Eneida R. Nemecek⁶, Kimberly Thormann¹, Kirk R. Schultz⁷, Georgia B. Vogelsang⁸

¹Robert H Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL, ²Levine Children’s Hospital, Charlotte, NC, ³University of Utah Health Sciences Center, Salt Lake City, UT, ⁴Methodist Children’s Hospital, San Antonio, TX, ⁵Dana Farber Cancer Institute, Boston, MA, ⁶Oregon Health & Science University, Portland, OR, ⁷University of British Columbia, Vancouver, BC, ⁸Sidney Kimmel Cancer Center of Johns Hopkins University, Baltimore, MD.

Corresponding Author:
David A. Jacobsohn, M.D.
Associate Professor of Pediatrics, Northwestern University School of Medicine
Stem Cell Transplant Program
Children’s Memorial Hospital
2300 Children’s Plaza, Box #30
Chicago, IL 60614
Phone 773-880-3694
FAX 773-880-3223
djacobsohn@childrensmemorial.org

Running Head Title: Pentostatin for refractory cGVHD in children
Abstract

There is no standard therapy for steroid refractory chronic graft-versus-host disease (GVHD). This problem is particularly daunting in children with chronic GVHD, where the effects of the disease and its treatment may impair normal growth and development. Children are also particularly vulnerable to failure and/or toxicity of therapy; for example, joint contractures or joint damage may result in life-long disability. The Pediatric Blood and Marrow Transplant Consortium performed a phase II trial of pentostatin for steroid refractory chronic GVHD in 51 children (median age 9.8 years) from 24 institutions. Overall response was 53% (95% CI 40%-64%), with a response of 59% (42%-75%) in sclerosis. Thirteen subjects (25%) had toxicity requiring them to stop pentostatin. The drug had a significant steroid-sparing effect in those that responded. There was also a trend towards increased survival at 3 years in responders vs non-responders (69% vs 50%, p=0.06). The IV administration of the drug ensures compliance in a patient group where oral therapy is difficult to monitor. Pentostatin has activity in refractory chronic GVHD in children and future studies, including treatment of newly-diagnosed children with high-risk chronic GVHD, are warranted. The trial was registered at www.ClinicalTrials.gov with identifier NCT00144430.
Introduction

Chronic graft-versus-host disease (GVHD) is the major cause of late morbidity and non-relapse mortality after hematopoietic stem cell transplant (HSCT). Pediatric chronic GVHD in particular remains an understudied area. The recovery of the pediatric immune system after HSCT is different than that of adults and stem cell source use in pediatrics is clearly different (e.g. more cord blood and less peripheral blood than in adults). Consequently response to immunosuppression may not be the same in children. Furthermore, children have many years to live post-HSCT and therefore minimizing irreversible changes of chronic GVHD such as joint contractures or pulmonary fibrosis is of paramount importance. The toxicity of long-term corticosteroid therapy is also a significant problem particularly for pediatric patients where the effects on bone and growth and development are more pronounced. Thus, there is a need for corticosteroid-sparing therapy in this group of patients.

Unfortunately, therapy for chronic GVHD has been associated with sub-optimal responses and significant toxicity and morbidity. Systemic corticosteroids with or without a calcineurin inhibitor remain the standard for initial therapy. However, many patients do not respond adequately and need further treatment. Recently reported salvage therapies include sirolimus, mycophenolate mofetil (MMF), rituximab, and extracorporeal photopheresis (ECP). Salvage studies in pediatric chronic GVHD have been small and limited mostly to MMF and ECP. MMF showed minimal response in what is considered a very difficult type of chronic GVHD to treat: sclerotic skin GVHD. Sclerotic GVHD in children has shown some response to ECP; however, it appears that this therapy is needed for a prolonged period of time to see an effect.
Furthermore, a number of these therapies have significant toxicities that can limit their utility, such as hemolytic-uremic-syndrome seen with sirolimus\textsuperscript{4} and significant diarrhea seen with MMF.\textsuperscript{9} Some therapies are more difficult in children. For example, ECP requires significant supportive care such as prolonged central venous line access and multiple transfusions throughout the length of treatment.\textsuperscript{10,11} Although compliance can be an issue with any age group, oral therapy can be particularly problematic for some children and young adults. Clearly, in a disease such as chronic GVHD there is need for a well-tolerated, easily administered and monitored therapy.

Pentostatin, a nucleoside analog that is a potent inhibitor of adenosine deaminase,\textsuperscript{12} has a broad spectrum of immunomodulatory activities. Most relevant to GVHD, this drug causes marked reduction of CD4 and CD8 cells. There is also significant B cell depletion with reduction of IgG levels.\textsuperscript{13} This should allow it to affect GVHD at the cellular level and thus has the potential to address the many manifestations of chronic GVHD. This is in contrast to other agents, such as imatinib\textsuperscript{14}, which targets selective pathways involved in fibrosis, and rituximab\textsuperscript{6}, which targets antibody production. Pentostatin was found to be active in a phase I study in refractory acute GVHD.\textsuperscript{15} A phase II study of pentostatin in heavily pre-treated patients (median age 33 years, median of four prior regimens) with chronic GVHD showed a fifty-five percent objective response rate in 58 patients.\textsuperscript{16}

Although responses to pentostatin in an adult population are very encouraging, that study was conducted at only two centers in a predominantly adult population. Because response in pediatric patients could theoretically be different than in older populations, we conducted multi-institutional study of pentostatin (deoxycoformycin
[Nipent]; Hospira, Inc., Lake Forest, IL) in pediatric patients with corticosteroid-refractory chronic GVHD to look both at response rate and to detect any unique toxicities in this age group.
Patients and Methods

Eligibility and enrollment

For all subjects, parental permission and subject assent, when applicable, was obtained in accordance with the Declaration of Helsinki. The study was approved by the institutional review boards of all participating PBMTC institutions. The trial was registered at www.ClinicalTrials.gov with identifier NCT00144430 on August 31, 2005.

In order to be eligible for study participation, subjects were required to have had an allogeneic stem cell transplant using any stem cell source and to be less than 21 years of age. In addition, subjects were required to have treatment-refractory chronic GVHD, defined as: a) development of one or more new sites of disease while being treated for chronic GVHD, b) progression of existing sites of disease while receiving treatment for chronic GVHD, or c) failure to improve despite at least 1 month of standard treatment for chronic GVHD. Standard therapy was defined as a regimen containing at least prednisone 1mg/kg every other day or equivalent dose of another steroid or another immunosuppressive regimen if patient was unable to tolerate steroid therapy. As eligibility was based on clinical criteria of chronic GVHD, subjects did not need to be more than 100 days following HSCT to be eligible. A tissue biopsy prior to entering study, with histology consistent with GVHD was required unless there was a medical contraindication such as concern for poor wound healing after the biopsy.

There were no restrictions regarding preparative regimen received or degree of HLA-mismatching. Myeloablative regimens were defined as those including busulfan ≥14mg/kg or total body irradiation ≥1200cGy prior to transplant. Reduced intensity included all others. HLA-typing reported was for HLA-A, HLA-B, and HLA-DRB1.
Patients who had failed more than two prior immunosuppressive regimens (in addition to their GVHD prophylaxis regimen) were not eligible. Patients with a forced expiratory volume in 1 second (FEV1) < 50% were also not eligible. Patients with a Karnofsky/Lansky performance score <40% were excluded, as were those with a calculated creatinine clearance < 30 ml/min/1.73m².

**Therapy**

Pentostatin was the only study intervention for the treatment of chronic GVHD for participants. Pentostatin was administered every two weeks at 4mg/m² by intravenous infusion over 20-30 minutes. An intravenous fluid bolus (5 ml/kg) was given before and after each dose. Dose was modified as follows: if the absolute neutrophil count (ANC) was below 500 cells/mm³ or the platelet count was below 20,000/mm³, or the estimated creatinine clearance was <50 ml/min/1.73m² and >30 ml/min/1.73m², the dose was reduced by 50%. If the estimated creatinine clearance was <30 ml/min/1.73m², pentostatin was held. Pentostatin was also held during severe infections at the discretion of the local PI. If subjects sustained a complete response at 6 months, pentostatin administration was stopped and subjects were followed for an additional 6 months to determine sustained response. All other subjects received 12 months of therapy unless they required earlier removal from the study.

It was recommended that a corticosteroid taper be started between 8-12 weeks after initiating pentostatin. A reduction of 25% of the initial dose every 2 weeks was the recommended corticosteroid taper. It was also recommended that if subjects were receiving a calcineurin inhibitor at the time of study initiation that they remain on it
through the duration of the study. All other immunosuppressants were to be tapered starting at 3 months.

The following infection prophylaxis was strongly recommended, but not required, for subjects on study: Trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* prophylaxis, antibacterial prophylaxis with penicillin, antiviral prophylaxis with acyclovir, and antifungal prophylaxis with fluconazole.

**Required evaluations and clinical endpoints**

Results of skin biopsies and/or additional pertinent organ biopsies were reviewed by the PI. At baseline and every two weeks, a history and physical were required, as well as complete blood count, chemistries, and liver function tests. A calculated creatinine clearance using the Schwartz formula was determined prior to each dose of pentostatin. Schirmer’s tests and pulmonary function tests were performed at baseline if the subject was old enough to cooperate and throughout the study as needed. Additional biopsies were performed when clinically indicated.

Subjects were evaluated at baseline and then every 3 months, using the form in Table 1, supplemental online material. The staging used in this study was very similar to the COG ASCT0031 response criteria (AG) and the NIH Consensus for Chronic GVHD staging recommendations, which were not yet available at the time this study started. Subjects were graded in nine domains. These domains combined reported symptoms and physical findings, on a scale of 0 to 3. Subjects were graded by each center’s investigator or designee. In addition, detailed symptom list and medication reports were submitted every 3 months. Domain responses were then determined based on the grading form. A
total of nine domains were assessed: rash, sclerosis, oral, fasciitis, joint, liver, lung, gastrointestinal, and ocular.

To obtain a response in a particular domain, the following criteria were used: For a partial response, there must be improvement in at least one of the items without worsening in any other item (i.e. for “rash” response a subject needed improvement in “rash” and/or “lichenoid changes” without worsening in other domains). For a complete domain response, a subject must be graded as “0” in all items in that domain at the subject’s last evaluation. Finally, a complete overall response was obtained by having all involved domains reach a complete response at the subject’s final evaluation, whereas a partial overall response represented partial response in any domain/s with no worsening in any other. No response was defined as progression, no change, or mixed response (improvement in one domain and worsening in another). Per the protocol, possibly irreversible manifestations (hypo/hyperpigmentation and ocular GVHD) were not used in determining overall response. Because of the subjective nature of ocular dryness, a score of 0 or 1 represented a complete ocular response, in a subject beginning with a score of 2 or 3. All subjects, regardless of number of pentostatin doses received, were evaluable for response. Overall response and organ-specific response were determined from the subject’s last evaluation, even if the subject was removed from study early (i.e. prior to 12 months) for toxicity, progression of chronic GVHD, or decision of the local PI or subject. Patients having an initial response and then a subsequent progression were considered non-responders. Thus, the overall response represents the best response except as stated above (response and subsequent progression was considered a non-
response). Subjects were not followed for response beyond the completion of the study (12 months).

Toxicity sheets were filled out on a monthly basis using Common Toxicity Criteria v3.0 (CTCAEv3.0). Non-hematologic toxicities grade ≥3 were reported in an expedited fashion. The reporting period for adverse events and toxicities was 28 days after the last on-study dose.

**Removal from Protocol**

Subjects were removed from study if they experienced disease progression after 3 doses of pentostatin or if they experienced a grade III-IV toxicity (related or not related to pentostatin) that was judged serious enough to preclude the subject from receiving further administration of drug. Subjects were also removed if they were lost-to-follow up or if family withdrew consent. For subjects that were removed from study, the last assessment prior to removal was used to determine response. Subjects that were removed prior to the first three-month evaluation for either toxicity or progression, and in whom a final assessment was not filled out (N=8), are counted as non-responders.

**Consensus Review, Statistical Considerations and Safety Monitoring**

A panel of six investigators (DJ, KT, EN, MG, LL, AG) convened for two days at the end of the study for response adjudication. Each member of the panel individually reviewed all case report forms and severe adverse events (SAE’s) for every subject. Domain response, overall response, and attribution of SAE’s were recorded by each member, and then each subject was reviewed by the panel. With regards to overall
response, there was unanimous agreement in 84% of cases and agreement by five out of the six experts in another 10% of cases. In the remaining 6% of cases, consensus was reached after discussion by the panel.

The primary statistical endpoint was overall response rate (defined as complete or partial remission). A Simon two-stage design was used to have 80% power to detect a response rate of at least 40%\textsuperscript{18} compared to a control response rate of 20%, with 80% power and a Type I error rate of 5%. While this design required 43 patients, a sample size of 50 was targeted while the final sample size was 51. The other major outcome of the study was determination of adverse events.

A secondary outcome was response in each domain, measured in subjects that had had initial involvement in that domain. Response rates were calculated together with 95% exact binomial confidence intervals. Descriptive characteristics are shown with percentages or medians and range, as appropriate. Fisher’s Exact Test was used to determine significance (judged as $p < 0.05$) of certain factors such as change in corticosteroid dose and impact of early response on final response. Time to initial response was analyzed using cumulative incidence where withdrawal due to toxicity was considered a competing event.\textsuperscript{19} Non-responders who were not withdrawn due to toxicity were considered to be censored at their last follow-up. Survival percentages and confidence intervals were calculated using Kaplan-Meier curves. Curves were compared using the log-rank test. All analyses were performed with SAS.\textsuperscript{20}

This study was overseen by the PBMTC Data Safety Monitoring Committee. Study reports were reviewed twice yearly. Reports included response and toxicity data. The study was designed to stop if it was determined early that it was futile treatment (if 3
or less of the first 13 subjects did not respond to treatment). The study continued to full accrual. The study was also overseen by the Food and Drug Administration under IND #67,084.
Results

Patient Characteristics

Fifty-one subjects from 24 different institutions were enrolled. Table 1 describes pre-transplant characteristics of the 51 subjects enrolled. Briefly, the median age at time of enrollment was 9.8 (0.9-20.7) years. Thirty-one subjects had received a HSCT for a malignant disease. Forty of the subjects had received an alternative donor stem cell source: unrelated cord blood (N=18), adult unrelated donor (N=19), and non-genotypically matched family member donor (N=3). Subjects had had chronic GVHD for a median of 6.2 (0.1-42.1) months prior to enrollment. Forty-seven of the subjects had had a tissue biopsy consistent with chronic GVHD and in four of the subjects the biopsy was not performed due to medical contraindication.

Table 2 describes the chronic GVHD characteristics of the subjects. Sixty-nine percent of subjects had had a history of prior acute GVHD, 34% had progressive-onset chronic GVHD (while on therapy for acute GVHD, they had progressed to chronic GVHD), and 59% had severe chronic GVHD according to the NIH Consensus global severity stage. Subjects had a variety of chronic GVHD manifestations at study entry (table 3), with skin being the most common (78% lichenoid changes/rash and 53% sclerosis). Most of the subjects with gastrointestinal manifestations had additional manifestations suggestive of chronic GVHD. However, four subjects had gastrointestinal symptoms as their major GVHD manifestations which could represent the late acute variant of GVHD. All subjects had received corticosteroids for treatment of chronic GVHD (median 3.9 (1-24.9) months), and 49 were on corticosteroids at study entry, with a median prednisone dose of 0.9 (0-2.4) mg/kg/day. Forty-five subjects had been on a
calcineurin inhibitor and 42 of them were on one at time of study entry. Other therapies
given prior to this study specifically for chronic GVHD included mycophenolate mofetil
(MMF n=30), infliximab (n=4), sirolimus (n=3), daclizumab (n=3), etanercept (n=2),
photopheresis (n=1), and hydroxychloroquine (or placebo) on COG ASCT0031, a study
from the Children’s Oncology Group open at that time (n=2).

Response

Overall, twenty-seven subjects had a response to treatment at their last evaluation,
for an overall response rate of 53% (40%-64%, 95% CI). Twenty subjects had a partial
response and seven had a complete response. These 27 subjects had an initial
documented response as follows: 23 at 90 days, 2 at 180 days and 2 at 365 days. All of
these 27 subjects sustained their response at time of last evaluation, which was at the
same time of initial response in 5 patients, at a range of 3 – 185 days after initial response
in 9 patients and 275 days after initial response in 13 patients. The median duration of
response was 185 days (range 0 – 275 days) after their initial response. For 20 subjects
the initial response remained the best response; however, seven continued improving
during their course of treatment. The last evaluation times were at 90 – 285 days in 11
responders and at 365 days in 16 responders. The median follow-up time in all 51
patients was 270 days (range 25 – 365 days). Twenty-four patients were non-responders.
Of these, 15 never showed a response and nine subjects had an initial or transient
response but later progressed. Figure 1 gives the cumulative incidence of time to initial
response where withdrawal due to toxicity is a competing risk.
Of the 51 subjects who entered the study, 21 received the intended 12 months of therapy. Removal from study prior to 12 months occurred for the following reasons: chronic GVHD progression (13), toxicity (13), relapse of malignancy (2), and loss to follow-up (2). Of the 21 subjects that received 12 months of therapy, 16 had an objective response (10 PR, 6 CR). Using the total sample size as the denominator, the percentage of patients who received 12 months of therapy and had a response was 16/51 (31%, 95% CI 20%-44%).

As for organ-specific response, among the 40 subjects with skin rash/lichenoid involvement, the response rate was 50% (36%-64%). Of the 27 subjects with sclerotic manifestations, 59% (42%-75%) showed response. All other manifestations and responses are detailed in table 3. No subjects with liver (N=5) or lung (N=2) manifestations had a response in these domains. Of the five subjects with liver GVHD, three were overall non-responders (2 of these had skin GVHD that worsened; one had liver GVHD as their only manifestation). One subject with liver GVHD was stable overall (liver GVHD was their only manifestation). The last subject with liver GVHD was an overall responder (the liver remained stable and rash/sclerosis improved). Of the two subjects with lung manifestations, one had stable lung involvement with improvement in rash and was classified as a responder whereas the other had worsening lung function with improvement in rash and was classified as a non-responder.

As secondary evaluations, we were interested in determining whether early response predicted overall response and ability to continue on study. There were 29 early responders (response by 3 months). Thirteen of those (45%) continued on study and maintained a partial or complete response at 12 months. There were 22 patients who
were not considered responders at the three month evaluation. Because the tempo of response was not known, some of these patients continued on study at the discretion of the investigator. Three of these 22 patients (14%) continued on study and achieved a partial (n = 2, initial response in both patients at 6 months) or complete response (n = 1, response at 12 months) (p=0.031 comparing 45% to 14%). Furthermore, we were interested in determining whether pentostatin provided a corticosteroid-sparing effect in subjects that responded. The initial median dose of prednisone in responding patients was 0.9 (0-2.4) mg/kg/day and at study end it was 0.2 (0-1.1) mg/kg/day (p<0.001). In the 24 subjects who did not respond to pentostatin, the initial median prednisone dose was 0.8 (0-2.2) mg/kg/day. Final dose was available for 15 of these non-responding subjects, and it was 0.9 (0-2.2) mg/kg/day. The change in prednisone dose in non-responding subjects was not statistically significant (p=0.89).

**Toxicity and Mortality**

Thirteen subjects went off-study due to toxicity. Table 4 summarizes the toxicities in these 13 subjects. Most adverse events occurred in the first 6 months. In fact, 11 of 13 patients discontinuing due to toxicity did so during the first 6 months of therapy. The most common adverse event was infection (N=27), which occurred in 15 subjects. Three subjects had ≥three infectious adverse events each and these accounted for 13 of the infectious adverse events. There were two cases of documented fungal and 3 cases of documented viral infections. The rest were documented or presumed bacterial infections requiring hospitalizations. All 3 subjects who died of infectious complications had sepsis with documented bacterial infections (methicillin-resistant *S. aureus*, *S.*
maltophilia, and P. aeruginosa). Autoimmune hemolytic anemia was seen in 3 patients. All three subjects were removed from study. With regards to malignancies, two subjects (out of 31 at risk) experienced relapse of their primary disease (leukemia). One subject developed EBV post-transplant lymphoproliferative disease.

Four subjects died while on therapy or within 28 days after last dose of pentostatin. Three of these deaths were due to infection and one was attributed to leukoencephalopathy in a subject previously diagnosed with reversible leukoencephalopathy secondary to tacrolimus. At one year, the overall survival of the entire cohort was 84% (74%-94%). At 3 years, the projected overall survival of the cohort was 60% (45%-75%). At three years after entry on to the trial, projected survival was 69% (95% CI 49%-89%) for patients who responded to pentostatin compared to 50% (95% CI 29%-71%) for those not responding (p=0.06), as shown in Figure 2. The median follow-up of survivors was 33 (range 18-60) months.
Discussion

In this phase II study we examined the response and toxicity of pentostatin in children with corticosteroid-refractory chronic GVHD. The study met its primary statistical endpoint, which was to show a response rate greater than 40%.

This study is important for a number of reasons. First of all, results in a pediatric population confirm the results seen in a prior phase II study of mostly adult subjects. The prior phase II study was performed at two sites and was thus subject to potential bias of limited-institution studies. Here 51 subjects were accrued from 24 sites, and an overall response of 53% was seen. The subjects in the current study represented a more homogenous population in terms of age and pre-treatment characteristics.

Secondly, while a number of studies have reported responses in this range in the salvage setting, most of the skin responses have been in the lichenoid/rash domains. A remarkable finding in our study is that we documented a 59% response rate in sclerotic manifestations. Of the nine subjects that started with 10%-50% sclerosis by BSA, seven had an overall response and of the three that started with >50%, two had an overall response. Sclerosis has been a manifestation of chronic GVHD that generally is less responsive to therapy and if it does improve tends to take a longer time compared to other manifestations.

In addition, we were also able to examine the tempo of response in this study, and showed that those subjects with a good response at 3 months were much more likely to continue improving and tolerate therapy for a longer period of time. This observation has important practical implications as one may consider not continuing therapy with pentostatin after 3 months if there has not been an objective response by that point. Early
response seems to be the most important predictor of overall outcome as we were unable to detect a difference in response rate based on multiple baseline patient characteristics such as steroid dose and platelet count (data not shown).

Finally, this multi-institutional study is the largest reported pediatric chronic GVHD clinical trial to date. The PBMTC brings together investigators from 70 North American institutions with an interest in clinical trials in pediatric hematopoietic cell transplantation. The enthusiastic completion of this study in a timely fashion demonstrates the feasibility of finishing studies of this size through the PBMTC and sets the stage for further studies in GVHD and supportive care after transplant.

There is also tremendous interest at this point in designing tools for staging and assessing response of chronic GVHD. The National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD (NIH Consensus Conference on cGVHD) initially convened in 2005 and since then there have been recommendations on what criteria to use in diagnosis, staging, and response.17,22 There is an ongoing cohort study prospectively studying the validity of these staging and response criteria (Dr. Stephanie Lee, Seattle, WA, personal communication), and a number of retrospective studies have looked at the applicability of these scales.23-25 The staging used in this study is very close to the NIH Consensus for Chronic GVHD staging recommendations.17 While the NIH Consensus recommends a different scale to measure response, we have shown within this study that the staging criteria is feasible to use in a multi-institutional setting and that response in this scale is consistent among a panel of graders. There was full agreement on response from the panel of graders in the majority of cases. Among the eight cases where there was not full agreement: in five of the cases
the majority graded the subjects as responders and in three of the cases the majority
graded the subjects as non-responders. Consensus was achieved with discussion and in
all eight cases it was the majority’s assessment of response which dictated the final
response assessment. Response was associated with objective, measurable and clinically
relevant endpoints. For example, response in this scale was associated with reduction in
immunosuppression, now considered one important endpoint in chronic GVHD
studies.26,27

Of course, one of the concerns in using a scale as we did, which is semi-
quantitative and combines signs and symptoms, is that it may not be sensitive enough to
detect all responses. It may not have the same level of correlation with response in a
group of patients with either a very high or a very low burden of GVHD signs and
symptoms. Also, the scale may not be completely applicable in an older population.
Hence the NIH Consensus has recommended a more quantitative scale, for patients of all
ages, to measure response.22 It is imperative that this scale be validated, an effort which
is currently under way in the ongoing cohort study described above. Additionally,
validating quality-of-life and symptom scales in pediatric patients with chronic GVHD
would add additional ways with which to measure response.

We show that along with a documented objective response and corticosteroid
reduction, pentostatin administration was simple and well-tolerated. The medication is
administered every 2 weeks in the span of 2-3 hours. Short term side effects were mostly
nausea and subjects were able to go back to their regular activities often the evening after
drug administration. One of the benefits of a drug with such a long pharmacodynamic
effect is this ability to dose it infrequently yet continue to have a prolonged benefit. In
chronic diseases where there is already a high burden of emotional and psychosocial issues, it would appear that simple therapies that require minimal efforts at home would be of some benefit. With an IV medication, monitoring of therapy is straightforward.

While this was not a randomized study, the toxicity encountered in this population does not appear to be disproportionate to what is seen with other therapies for patients with corticosteroid-refractory chronic GVHD. For example, the recently published experience with imatinib had a similar rate of removal from study for toxicity (7/33=21%) as with this study (13/51=25%). Most of the toxicities in the current trial occurred early and were infectious in origin. It is hard to know if the infections were due to the underlying disorder (the immunodeficiency of chronic GVHD), the cumulative toxicity of prior therapies, and/or the experimental therapy being tested. Baseline factors were not significantly associated with infectious adverse events (data not shown).

However, there was a trend towards more infectious adverse events in subjects with a history of acute GVHD (p=0.06). With respect to other studies, most reports of salvage approaches are either too small or target a very specific patient type which makes a comparison of infectious mortality difficult. The infectious mortality (3/51) encountered in our study does not appear to be significantly different to what was seen in the randomized study where MMF was added to standard therapy in newly-diagnosed patients with chronic GVHD. In that study, three of 74 subjects on the MMF arm died of infection.

One toxicity deserving mention is autoimmune hemolytic anemia which occurred in 3 subjects and was severe enough to require the subjects to go off-study and receive treatment with rituximab. Autoimmune hemolytic anemia following HSCT has been
described both in adults and children,\textsuperscript{30,31} and one can hypothesize that the cause is the immune dysregulation from chronic GVHD, from the therapy for chronic GVHD, or both. Future chronic GVHD studies should be able to better separate the direct effects of the drug on the immune system from the underlying disease and its prior therapies. The current resurgent interest in the basic biology of chronic GVHD and correlative studies done in these patients\textsuperscript{32,33} will hopefully allow this level of immune dissection in the future.

While long-term outcome was not a primary outcome of the study, the 3-year projected overall survival of 60\% is encouraging. There are very few publications reporting the long-term outcome of chronic GVHD in children\textsuperscript{34} and even less data regarding the long-term outcome of children with chronic GVHD needing salvage therapy. In a retrospective Italian Association for Pediatric Hematology and Oncology (AIEOP) study, the 3-year disease-free-survival of a large cohort of newly-diagnosed children with chronic GVHD was about 70\%.\textsuperscript{34} The 3-year survival of 60\% in our study is therefore encouraging, given all had failed initial conventional therapy. Likewise, the trend towards improved survival in patients responding to therapy (versus those not responding) with pentostatin is heartening. Therefore one can view response as a potential surrogate marker for long-term survival. This observation is consistent with other studies where complete or partial response of the chronic GVHD is associated with less non-relapse mortality and thus increased survival.\textsuperscript{7,35}

In conclusion, we have demonstrated, in a multi-institutional study, a response rate of 53\% in pediatric patients treated with pentostatin for corticosteroid-refractory chronic GVHD. Toxicity was acceptable and long-term survival is encouraging. We
strongly support future multi-institutional studies to be performed through the PBMTC. We also recommend the study of pentostatin in patients with newly-diagnosed, high-risk chronic GVHD.
Acknowledgements:

The authors acknowledge all members (physicians and research nurses) of the Pediatric Blood and Marrow Transplant Consortium (PBMTC). The members are listed on the website www.pbmtc.org. A complete list of participating institutions and principal investigators is included in the supplemental appendix.

This work was an investigator initiated study (DAJ) supported by a grant from SuperGen, Inc. and Hospira, Inc.

Authorship:

Contribution: DAJ and GBV wrote the protocol and the manuscript and analyzed data. ALG and AR contributed to the protocol design. AR provided statistical support and wrote parts of the manuscript. KRS provided scientific support and helped analyze the data. BB, MG, LL, ERN, KT participated in data collection. All authors contributed to writing the paper, and checked the final version.

Conflict-of-interest disclosure: DAJ received a research grant from SuperGen, Inc. and Hospira, Inc. for this study and has received honoraria as part of speakers' bureau from them.
References


<table>
<thead>
<tr>
<th>Table 1. Patient characteristics at study entry. N=51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (median, range)</td>
</tr>
<tr>
<td>Months from transplant to GVHD (median, range)</td>
</tr>
<tr>
<td>Months from GVHD to study entry (median, range)</td>
</tr>
<tr>
<td>Months from transplant to study entry (median, range)</td>
</tr>
<tr>
<td><strong>Gender:</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Diagnoses:</strong></td>
</tr>
<tr>
<td>Acute myeloid leukemia/Myelodysplasia</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
</tr>
<tr>
<td>Metabolic disorder</td>
</tr>
<tr>
<td>Hemoglobinopathy</td>
</tr>
<tr>
<td>Immune deficiency</td>
</tr>
<tr>
<td>Bone marrow failure syndrome</td>
</tr>
<tr>
<td>Familial hemagophagocytic lymphohistiocytosis</td>
</tr>
<tr>
<td>Transplant regimen:</td>
</tr>
<tr>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>Myeloablative</td>
</tr>
<tr>
<td>Reduced intensity/reduced toxicity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stem cell source:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-identical sibling bone marrow</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>HLA-identical sibling PBSC</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>HLA-identical sibling cord blood</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Unrelated cord blood†</td>
<td>18 (35%)</td>
</tr>
<tr>
<td>Unrelated donor bone marrow††</td>
<td>9 (17%)</td>
</tr>
<tr>
<td>Unrelated donor PBSC†††</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Non-genotypically matched family member donor bone marrow (HLA 6:6)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Non-genotypically matched family member donor PBSC††††</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

†HLA-matching: HLA 4:6 (N=5), HLA 5:6 (N=11), HLA 6:6 (N=2)
††HLA-matching: HLA 5:6 (N=1), HLA 6:6 (N=8)
†††HLA-matching: HLA 5:6 (N=4), HLA 6:6 (N=6)
††††HLA-matching: HLA 5:6 (N=1), HLA 6:6 (N=1)
Table 2. Chronic GVHD Characteristics at Study Entry

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning platelet count (median, range)</td>
<td>267 (16-683 x 10^3/mm^3)</td>
</tr>
<tr>
<td>Beginning prednisone dose (mg/kg/day) (median, range)</td>
<td>0.9 (0-2.4)</td>
</tr>
<tr>
<td>Months on corticosteroids prior to entry (median, range)</td>
<td>3.9 (0-24.9)</td>
</tr>
<tr>
<td>Subjects on corticosteroids at study entry</td>
<td>49 (96%)</td>
</tr>
<tr>
<td>Subjects with platelets under 100,000</td>
<td>10 (20%)</td>
</tr>
<tr>
<td>Performance score &lt;= 80</td>
<td>19 (37%)</td>
</tr>
<tr>
<td>History of acute GVHD</td>
<td>35 (69%)</td>
</tr>
<tr>
<td>Progressive onset chronic GVHD</td>
<td>17 (34%)</td>
</tr>
<tr>
<td>Severe chronic GVHD (NIH global severity stage)</td>
<td>30 (59%)</td>
</tr>
<tr>
<td>Tissue biopsy consistent with GVHD</td>
<td>47 (92%)</td>
</tr>
</tbody>
</table>
Table 3. Response by domain

<table>
<thead>
<tr>
<th>Domain</th>
<th>Involved (% of 51)</th>
<th>Complete</th>
<th>Partial</th>
<th>Stable</th>
<th>Worse</th>
<th>Response Rate (CR+PR (95% CI))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash/Lichenoid changes</td>
<td>40 (78%)</td>
<td>13 (33%)</td>
<td>7 (17%)</td>
<td>10 (25%)</td>
<td>10 (25%)</td>
<td>50% (36%-64%)</td>
</tr>
<tr>
<td>Sclerosis</td>
<td>27 (53%)</td>
<td>11 (41%)</td>
<td>5 (18%)</td>
<td>6 (22%)</td>
<td>5 (19%)</td>
<td>59% (42%-75%)</td>
</tr>
<tr>
<td>Oral</td>
<td>30 (59%)</td>
<td>9 (30%)</td>
<td>8 (27%)</td>
<td>7 (23%)</td>
<td>6 (20%)</td>
<td>57% (40%-72%)</td>
</tr>
<tr>
<td>Fasciitis</td>
<td>7 (14%)</td>
<td>2 (29%)</td>
<td>1 (14%)</td>
<td>2 (29%)</td>
<td>2 (29%)</td>
<td>43% (18%-71%)</td>
</tr>
<tr>
<td>Joint</td>
<td>20 (39%)</td>
<td>5 (25%)</td>
<td>4 (20%)</td>
<td>5 (25%)</td>
<td>6 (30%)</td>
<td>45% (27%-64%)</td>
</tr>
<tr>
<td>Liver</td>
<td>5 (10%)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0%</td>
</tr>
<tr>
<td>Lung</td>
<td>2 (4%)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>21 (41%)</td>
<td>8 (38%)</td>
<td>1 (5%)</td>
<td>7 (33%)</td>
<td>5 (24%)</td>
<td>43% (25%-62%)</td>
</tr>
<tr>
<td>Ocular</td>
<td>28 (55%)</td>
<td>5 (18%)</td>
<td>2 (7%)</td>
<td>13 (46%)</td>
<td>8 (29%)</td>
<td>25% (13%-41%)</td>
</tr>
</tbody>
</table>
Table 4. Adverse events which required subjects to go off-study

<table>
<thead>
<tr>
<th></th>
<th>Month on study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-6</td>
<td>6-12</td>
</tr>
<tr>
<td>N of subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Documented bacterial requiring infection</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Presumed bacterial infection</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Documented fungal infection</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Documented viral infection</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Central nervous system (leukoencephalopathy)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal/liver (pancreatitis/abdominal pain)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Hematologic (Autoimmune Hemolytic Anemia)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Subjects with adverse events that required them to be removed from study, separated by two 6-month time periods on the study.
Figure Legends:

Figure 1. Cumulative incidence of initial response. Withdrawal due to toxicity is considered a competing event. Number at risk = number of patients still on study at that time point who have either a) not had an initial response or b) not been withdrawn for toxicity or c) not been censored.

Figure 2. Overall survival. Individual curves shown for subjects that had response as compared to those that did not.
Number at Risk:

51  41  15  9  7

Figure 1.
Figure 2.
Evaluation of pentostatin in corticosteroid-refractory chronic graft-versus-host disease in children: a Pediatric Blood and Marrow Transplant Consortium study

David A. Jacobsohn, Andrew L. Gilman, Alfred Rademaker, Brittna Browning, Michael Grimley, Leslie Lehmann, Eneida R. Nemecek, Kimberly Thormann, Kirk R. Schultz and Georgia B. Vogelsang