



**Canadian Blood and Marrow Transplant Group
Graft Versus Host Disease Symposium
October 3rd, 2009, Montreal, QC**

The CBMTG would like to thank Genzyme for their exclusive support of this symposium.



The first graft versus host disease (GVHD) symposium was held in Montreal on October 3rd, 2009. It was organized by the Canadian Blood and Marrow Transplant Group (CBMTG) with the support of an unrestricted educational grant from Genzyme Canada. The meeting brought together clinicians and researchers from every bone marrow transplant centre across Canada. During his opening remarks, Jean Roy MD, FRCPC, chair of the symposium, thanked everyone for attending and welcomed them to the city. “Today’s symposium started as a vague idea in 2008,” he said, adding that it became reality through the help of Genzyme Canada. He also thanked the meeting planners. The symposium qualifies as a level two self-learning assessment with the Royal College of Physicians.

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Review of Acute GVHD Pathophysiology

Claude Perreault MD, FRCPC

Graft versus host disease (GVHD) is directly related to the ability of the immune system to discriminate between self and non-self. The immune system is divided into two major branches, innate and adaptive. The innate immune system is nonspecific and uses a limited number of receptors, mostly toll-like receptors (TLRs) that react to invading pathogens by releasing inflammatory cytokines. In contrast, the adaptive immune system is targeted, involves many receptors, and acts through both intracellular (i.e., T-lymphocytes) and extracellular (i.e., antibodies) pathways. In acute GVHD (aGVHD) donor T-lymphocytes attack the recipient.

The first step of aGVHD pathophysiology, activation of cytoreductive conditioning, is well-known. The second step is the induction in secondary lymphoid organs. Following interaction with host antigen-presenting cells (APCs), donor T-cells expand and differentiate into activated T-cells. Target organ damage is caused by inflammatory cytokines released from activated T-cells that have infiltrated tissues. Both innate and adaptive systems work together and can be disrupted in the setting of GVHD. Recognition of host antigens occurs in the secondary lymphoid organs. Blocking access to these priming sites may also block aGVHD. However, blocking entry to selected organs with the monoclonal antibodies MAdCAM1 has not prevented aGVHD in preclinical models. Access to all organs would need to be blocked.

The pattern of aGVHD differs between recipients. This may be due to different tissue distribution of minor antigens (MiHAs) recognized by the attacking T-cells. Alloreactive T-cells migrate to the bone marrow and thymus of the recipient. Thymic activity may be a predictor of GVHD. Recently, the presence of protein tyrosine kinase 7 (PTK7) in CD4 T-cells has been identified as a reliable marker of thymic function in allogeneic recipients.

The most important marker for predicting GVHD is the HLA antigen mismatch. Other minor antigens have also been identified; however, evidence suggests that a single MiHA is insufficient to cause GVHD. This may change since researchers may not have identified key MiHAs antigens in GVHD. Immunizations against single MiHAs may offer a therapeutic

alternative since mice injected with anti-H7^a T-cells did not develop GVHD. Donor gene expression profile may be predictive of aGVHD. Patients receiving transplants from donors with high levels of *SMAD3* do not develop GVHD; however the data is still too preliminary to be used clinically. Finally, selective depletion of alloreactive T-cells should inhibit aGVHD. As well, T_{reg} cells will also suppress a T-cell reaction. Currently, methods for *in vitro* expansion of antigen-specific T-cells do not exist. This needs to be an area of focussed research.

Following the presentation, a participant asked about the use of rituximab as an anti-B-cell possibility. Perreault responded that it is important to discriminate between acute and chronic GVHD (cGVHD). B-cells play a larger role in cGVHD; however, in aGVHD, B-cell depletion is unlikely to be efficient.

Evaluation of NIH Consensus Criteria for Classification of Acute and Chronic GVHD

Paul J. Martin, MD

Originally identified as late GVHD in the 1970s, chronic GVHD (cGVHD) is a pleomorphic syndrome affecting multiple organs with an onset typically 2 to 24 months post-transplant. Between 40 to 60% of 3-month survivors will develop the condition, which requires prolonged immunosuppressive treatment. Compared to acute GVHD (aGVHD), it starts later, lasts longer, and has different clinical manifestations. In 1980 the definition of cGVHD was expanded to include 'limited' and 'extensive' classifications, however, this classification proved to be inadequate. Many cases didn't clearly fit into either category, and some cases of cGVHD seemed to completely mimic aGVHD. To help simplify differentiation, the Seattle Group revised the definitions in 2001 based on the need for systemic immunosuppressive treatment. In 2004, the National Institutes of Health (NIH) attempted to establish common criteria and language for clinical trials. Diagnostic manifestations of cGVHD were divided into skin, mouth, GI tract, and other signs, as well as a set of distinctive manifestations that require biopsy to support the diagnosis. The criteria also describe a set of overlap manifestations found in both acute and cGVHD. The NIH introduced a new sub-category of cGVHD called 'overlap syndrome,' characterized by manifestations typically associated with acute GVHD. Clinical manifestations of cGVHD, including overlap syndrome, can begin before or after day 100. Acute GVHD is subdivided into classic acute disease with onset before day 100, and persistent or late-onset aGVHD.

Several retrospective analyses have investigated whether the NIH distinction can predict outcome. In general, results have not been consistent. This may be due to the differences in historical definitions of cGVHD or inadequacies documenting the disease manifestations in patient medical records. Late-aGVHD may have a shorter time to resolution compared to chronic disease, but beyond that, no differences have emerged. This result suggests that patients with late-aGVHD and NIH-defined cGVHD can be enrolled together in clinical trials; nonetheless, the two groups should be stratified within clinical protocols. The NIH criteria categorize aGVHD based on number of organs involved and severity of involvement; however,

it is not clear if organ severity at onset impacts survival. Risk factors for developing aGVHD and cGVHD appear to be similar with two exceptions: Older patient age and use of growth factor-mobilized blood cell grafts were each associated with an increased risk for cGVHD but not acute GVHD. Finally, in cGVHD, short-term response does not appear to be predictive of cure. Martin suggested that in future studies, response and tolerance should be distinguished from each other.

In the discussion that followed, Martin indicated that late-aGVHD and cGVHD probably have different biological manifestations. He believes that immunosuppressants may mask cGVHD, which becomes unmasked once immunosuppression is withdrawn. In response to a question about physician adherence to the NIH definitions, Martin said that the biggest threat to meaningful clinical trial results is inappropriate over-treatment and misdiagnosis of cGVHD by clinicians who initiate treatment too quickly. Other discussion points included the role of biopsy in confirmation of cGVHD, data on outcomes for patients with bronchiolitis obliterans, and tapering strategies for immunosuppressive regimens.

The Use of ATG to Prevent Acute GVHD

James A. Russell, MD

Graft versus host disease (GVHD) is still a major challenge in allogeneic transplant, occurring in 25 to 50% of unrelated donor transplant recipients receiving prophylaxis with methotrexate and cyclosporin (Mtx/CSA). Anti-thymocyte globulin (ATG) at appropriate dosing has been shown to reduce acute GVHD (aGVHD) incidence; however, it is also associated with higher rates of relapse, infection, and post transplantation lymphoproliferative disorders (PTLPD). Four ATG preparations are commonly used. In Canada, the use of Thymoglobulin in unrelated donors started around 1995. In studies, unrelated donor transplant recipients treated with ATG had a lower incidence of aGVHD compared to untreated recipients. Overall outcomes (overall survival, disease-free survival, relapse, treatment-related mortality) were similar in both groups. Although many studies didn't show a survival benefit, none showed a survival disadvantage. "It may come down to a question of quality of life," said Russell. Only a few studies exist in donor-related transplants, however, results seem similar.

The Alberta Blood and Marrow Transplant Program has been using Thymoglobulin in all stem cell transplants since 1999, mostly with the FLUBUP/Thymoglobulin/TBI regimen. Thymoglobulin is administered at 4.5 mg/kg over 3 days, finishing at day 0. In an analysis of matched related donors using this regimen, Russell et al. reported a trend toward reduction in aGVHD, and a significant reduction in transplant-related mortality. This appeared to be due to a reduction in GVHD-related death, including opportunistic infections. A trend toward increased relapse was observed in patients who received Thymoglobulin, however, overall survival was significantly improved. To validate the results of this single-centre study, the CIBMTR performed a retrospective case-control analysis in matched pairs. Similarly, aGVHD and overall mortality were significantly reduced compared with matched controls, and a trend toward increased relapse was observed. The difference in findings for aGVHD may be due to power of the study.

Bone marrow recipients appear to have a lower incidence of aGVHD compared to blood cell recipients. For unrelated donors, the difference is significant. Dosing and timing of Thymoglobulin administration is also important. A lower incidence of aGVHD has been

observed when smaller doses of ATG are administered closer to transplant. This approach is also less expensive. Finally, based on data from a Swedish study, low serum levels of r-IgG may predict aGVHD in patients receiving Thymoglobulin.

Challenges to the use of ATG which could require protocol modification include relapse, GVHD and opportunistic infections. The addition of two doses of total body irradiation (TBI) to the FLUBUP regimen has been shown to increase disease-free survival by reducing relapse. No effect on transplant-related mortality (TRM) was observed. Older high-risk patients appear to be at the greatest risk of non-relapse mortality (NRM). This appears to be due to increased incidences of aGVHD, chronic GVHD (cGVHD), and death related to GVHD. Patients with progressive cGVHD have poorer survival outcomes compared to those with non-progressive disease. Opportunistic infection in fact is most troublesome in patients treated for GVHD so it is doubtful whether reducing ATG dose would help in this respect.

Following the discussion, several participants asked whether data exist for younger patients. Dr. Russell replied that adults younger than 45 years have non-relapse mortality of about 5% after matched related transplants and data in children are also encouraging. Another participant asked about incidence of PTLPD, which Russell confirmed as being a concern that needs addressing. Participants also discussed if a controlled study with related transplants was warranted at this time. Most agreed it would be of interest.

Acute GVHD Treatment Options: Fact and Controversy

Silvy Lachance MD, FRCPC, CSPQ

Despite prophylaxis aimed at preventing acute GVHD (aGVHD), it still remains a major cause of morbidity and mortality. Between 30 to 50% of transplant recipients from a related donor will experience moderate to severe aGVHD. The incidence in unrelated transplants is even higher. Response rates and 100-day survival rates decrease with increasing severity of acute GVHD. Non-selective T-cell depletion is the most effective way of preventing aGVHD, however, it is associated with higher graft failure and relapse rates. For treatment, corticosteroids remain the most effective choice of primary therapy, with response rates between 40 to 50%. Dosing is currently lower than what was used in the 1990s as a result of studies showing that response rates in patients receiving low-dose were similar to those receiving high-dose steroid.

Several predictors of transplant-related mortality (TRM) have been identified among responders to steroid treatment, including older age, aGVHD \geq grade II, non-response by day 5 of treatment, advanced disease, and matched unrelated donor (MUD) transplant. When these predictors were used to stratify risk, a significant relationship was observed between risk category and TRM or overall survival. Among non-responders, predictors of TRM include older age, gut or liver GVHD, use of ATG second-line, and advanced disease. Responders who receive ATG have better outcomes overall than non-responders. Lachance said that 5-day response may help identify patients with different risks of TRM.

Increasingly, combined modality treatments (CMT) are being investigated to treat aGVHD. The combination of prednisone 1 mg/kg/day and beclomethasone has been shown to significantly reduce treatment failure and mortality in patients with gastrointestinal GVHD and without skin or liver involvement. In contrast, addition of daclizumab to 2 mg/kg/day prednisone in patients with aGVHD was associated with reduced 100-day and overall survival, increased risk of relapse, and increased risk of infection. "Daclizumab does not have a role in treatment," said Lachance. A more recent study investigating the addition of etanercept, mycophenolate mofetil (MMF), denileukin diftitox, or pentostatin to methylprednisone concluded that the MMF was the most promising addition to corticosteroid regimen. A phase 3 clinical trial is expected to start soon.

Based on the available evidence, Lachance proposed a treatment algorithm for patients. For patients who develop aGVHD, grade I-IIa, treatment should be initiated with prednisone at 1mg/kg/day. Patients with GI manifestations benefit from the addition of oral beclomethasone. Topical treatments should also be considered where appropriate. Patients diagnosed with grade IIb or higher should be treated with methylprednisone 2 mg/kg/day. Treatment response at day 5 will help predict TRM and survival. Innovative approaches should be considered for patients with grade III or IV disease, including CMT and cellular therapy. Supportive measures are important for all patients, and play a key role in quality of life. Patients on high doses of steroids need to be monitored closely for cytomegalovirus (CMV) and fungal infections.

Following the presentation, participants inquired about research predicting aGVHD prior to onset of symptoms, such as biomarkers. Lachance said that this is a very “hot topic,” however, she doubted that identifying these patients earlier would translate into better outcomes at this point. There was also significant discussion among participants revolving around initiating a randomized pilot study investigating surrogate markers, or addressing how markers could be incorporated into treatment.

Steroid Refractory Acute GVHD: And Now...?

Daniel Couriel, MD

Steroids still remain the primary therapy for the treatment of GVHD, however, it is unclear when a patient should be considered steroid refractory (SR). The current definitions range from the exhaustively detailed to ambiguously broad. Outcomes in acute GVHD (aGVHD) are worse among non-responders to primary therapy. Unfortunately, only a few well-designed clinical trials have assessed the effectiveness of therapies currently being used for SR-aGVHD. Furthermore, these studies frequently differ in their definition of response criteria and steroid refractoriness. Polyclonal antibodies like ATG are the most common choice of therapy. Efficacy rates range between 30 to 54%, however, efficacy and potency vary. The best organ responses are obtained in skin and GI-only disease. Liver response is poor. Toxicities are infusion related. Survival rates are also poor, between 10 to 20%.

Several options with biologic therapies have been investigated, including monoclonal antibodies, immunotoxin conjugate, and TNF-alpha blockade. Daclizumab is a humanized anti-CD25 antibody. It appears highly effective, but it is also associated with high mortality, mainly from infectious causes. Alemtuzumab is an anti-CD52 antibody administered subcutaneously. In a small study it demonstrated good response (OR: 83%, 33% CR) and survival rates, especially in patients with GI involvement. Responses were also observed in patients with liver GVHD. Denileukin diftitox is a recombinant protein of IL-2 fused to the diphtheria toxin. Overall response and complete response rates were relatively high in a dose-finding study. Dose-limiting toxicities were transaminase elevation and infection. In one study, TNF-alpha blockade with infliximab has demonstrated high response rates (OR: 67%, CR 63%), especially in patients with GI GVHD. However, most patients in this study had grade II aGVHD. The main toxicity was infection. Etanercept is a fusion molecule of TNF-alpha and IgG1. In a small study, overall response and complete response were also significant. Some reports suggest that it may have added benefit when added to daclizumab or ATG.

Mycophenolate mofetil (MMF) is widely used in GVHD mostly in the prophylaxis of chronic GVHD. In SR-aGVHD, Furlong et al reported overall response rates of 47%; however, these

results are difficult to interpret since the AUC may not have been optimized. In a small study, pentostatin demonstrated good response rates (78% RR, high CR 64%), including cases of liver GVHD. Dose-finding studies suggest that it is ineffective at doses below 1.5 mg/m²/day for 3 days. Extracorporeal photopheresis (ECP) and mesenchymal stem cells (MSC) are newer approaches. Response rates with ECP are good, with the better results observed in patients with shorter intervals from diagnosis of chronic GVHD to ECP. Survival rates were good for ECP responders, but not non-responders. Best responses were observed in skin and liver acute GVHD. Overall and complete response rates were promising in initial studies of SR-a GVHD. Again, mortality is significantly lower in responders versus non-responders.

In the absence of clinical trials, Dr Couriel recommended an organ and evidence-based approach. For aGVHD involving the skin, consider ATG, daclizumab, or photopheresis. For GI involvement, consider infliximab or ATG, and for SR aGVHD of the liver, photopheresis, pentostatin, or alemtuzumab are valid alternatives. Dr Couriel also suggested that the experience of the treating center with a particular immunomodulator is also an important factor to consider when choosing any particular form of GVHD therapy. . Supportive and ancillary care is also important in SR-aGVHD. This includes but is not limited to: wound care, the use of steroid-sparing agents and infectious prophylaxis where appropriate, and the diagnosis and management of treatment-related complications.

Following the presentation, discussion centred around the difficulties of interpreting clinical trials since patient populations and dosing schedules are not always consistent. Participants also discussed the result of Osiris' studies, which Couriel described as "very compelling."

A Newly-Funded Study: An RCT of Thymoglobulin in Unrelated Donor BMT

Irwin Walker, MBBS, FRACP, FRCPC

Walker announced that the CIHR has agreed to fund a new randomized clinical trial of Thymoglobulin in the prevention of chronic GVHD (cGVHD). He acknowledged the people involved in putting together the proposal, including Jim Russell, the CBMTG, and Genzyme. The project came about following results from studies suggesting less cGVHD in patients receiving ATG. As these results were not of the primary endpoints, which were of acute GVHD, more research was needed.

In this newly-approved study, the primary endpoint will be freedom of cGVHD at 12 months from transplantation defined as withdrawal of all systemic immunosuppressive agents without resumption up to 12 months after transplantation. The 12-month time point was chosen because most cases of cGVHD occur within the first 12 months post-transplant. As well, previous clinical trial results suggest that those who develop cGVHD will probably still be receiving treatment at this time.

The investigators are hypothesizing that the addition of Thymoglobulin will result in a decrease in the proportion of patients requiring immunosuppressive treatment for cGVHD without affecting all-cause mortality, non-relapse mortality, relapse rate, or infectious deaths. Walker added that the ultimate goal of the study is to decrease the suffering of patients post-transplant, and to reduce the number of patients in clinics. An enrollment of 162 patients is needed to adequately power the study. One of the main challenges to accrual may be the increased use of ATG in Canada over the last two years.

Inclusion criteria are adults receiving transplantation from an unrelated donor that is either fully MHC matched with the recipient or is 1-antigen or -allele mismatched at the A, B, C or DRB1 loci. Investigators are still deciding if children or cord blood donors can be enrolled. GVHD is lower in these populations and this may impact the power the study. For the protocol, patients will receive usual institutional preparative regimens with Thymoglobulin added to the active treatment arm for three days starting at day 2 before transplant. An attempt will be made to maintain preparative regimens at the varying centres; however, participating centres will need to

submit their regimens before starting enrollment. Supportive management will also be according to individual institutional protocols.

Next steps will be to get the off-label use for the study approved by Health Canada, assemble an ethics board, and starting working with a CRO. Walker said he foresees starting recruitment in April 2010. He anticipates several published papers to come out of this study.

Following the presentation, there was significant discussion around the 12-month endpoint, tapering protocols, and including related donor transplants. The main debate focussed around whether patients would still be on treatment at this time. Walker said this endpoint will be reinforced by positive effects on quality of life to demonstrate that the need for steroid therapy is meaningful to patients. He added that the protocol will likely have provisions for an interim analysis. He said that including related donors in the protocol may help improve accrual; however, ultimately this is a different patient population and so this amendment will be held in reserve. If accrual becomes a problem the preference would be to recruit additional centres from outside Canada to enrol unrelated donor transplants.