BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

FREQUENTLY ASKED QUESTIONS (FAQs) FOR BMT CTN PROTOCOL #0303

1. Why conduct a transplant trial evaluating T cell depletion in acute myeloid leukemia (AML)?

Patients with acute myeloid leukemia under the age of 61 who achieve a complete remission (CR) following induction chemotherapy require consolidation treatment in order to maintain remission and survive long term. There are several approaches to consolidation including multiple cycles of high dose cytarabine, autologous stem cell transplantation (AutoSCT), and allogeneic stem cell transplantation (AlloSCT) if a suitable donor can be identified. Based on the results of numerous national and international cooperative group trials, the karyotype at initial diagnosis is the strongest predictor of relapse risk and is currently used to assign individuals to a particular consolidation strategy. Patients with intermediate or high-risk cytogenetics have between a 60-90% risk of relapse following conventional chemotherapy-based consolidation. Strategies to limit relapse are needed in these patients. It remains controversial whether AutoSCT results in a meaningful improvement in disease free survival (DFS) compared to high dose cytarabine-based consolidation as the results of randomized trials have differed. Relapse rates are clearly lowest following AlloSCT, but any potential survival advantage has been mitigated by high rates of mortality related in large measure to acute graft-versus-host disease (GVHD) and complications related to its treatment. Several single center trials have developed various forms of T cell depletion (TCD) as a strategy to prevent or limit GVHD following HLA-identical sibling transplantation for AML in CR1 or CR2. The results have been quite encouraging, with reported DFS rates between 60-75% for AML in CR1 and >55-60% in CR2. Such results warrant further investigation and ultimately comparison to more conventional pharmacological approaches to prevent GVHD. However, before embarking on a large and resource intensive Phase III randomized trial of T cell depletion versus traditional pharmacologic GVHD prophylaxis, it is necessary to first confirm the encouraging results obtained in these single center trials as well as work through potential logistical hurdles. This can best be accomplished via a smaller multi-center Phase II trial involving a limited number of centers with significant experience performing AlloSCT and TCD in patients with AML. If the encouraging data can be confirmed, a larger Phase III trial conducted by the BMT CTN would be proposed.

2. How was the conditioning regimen chosen?

The conditioning regimen proposed for this trial incorporates fractionated total body irradiation (TBI) (1360-1375cGy) along with thiotepa, cyclophosphamide, and antithymocyte globulin (ATG). This regimen was developed at Memorial Sloan Kettering Cancer Center (MSKCC) in a series of studies conducted over the last decade and has resulted in a lowering of the incidence

of graft failure in recipients of T cell depleted transplants to < 2%. It is associated with a low risk of extramedullary toxicities and is well tolerated by adult recipients. The Perugia group has also published its experience using this regimen in AML. Together, the two centers have performed over 90 T cell depleted transplants in patients with first or second remission AML. Thus, this is the most extensively studied conditioning regimen in adult patients with AML undergoing T cell depleted transplants. The very low rates of graft failure and -elated mortality observed warrant further exploration of this regimen in a multi-center Phase II trial.

3. How were the eligibility criteria determined?

Patients with AML who achieve a first complete remission are at a significant risk for relapse despite the use of conventional consolidation chemotherapy. Exceptions to this generalization include patients with acute promyelocytic leukemia (APL)(FAB M3) who can remain in remission with chemotherapy combined with all-trans retinoic acid (ATRA) as well as patients with better risk karyotypes such as t(8;21) and inv16 or t(16;16). These better risk patients will be excluded from the trial if they are in first remission since they do not clearly benefit from allogeneic transplantation. Patients with t(8;21) or inv16 or t(16;16) will be eligible for the trial only if they are in second complete remission. All other groups of patients with AML under age 61 years and with an HLA-matched sibling donor available will be eligible due to the 40-90% risk of relapse even following high dose cytarabine-based consolidation. Patients with a normal karyotype are eligible in first remission since they still have a 40-70% risk of relapsing, particularly if they present with a high white blood cell count or have a mutation of the Flt-3 gene. Patients are eligible for this procedure in second remission since virtually all patients with AML, with the exception of patients with APL, will relapse without a transplant even after achieving a second remission.

4. Why was $CD34^+$ cell selection chosen as the method of T cell depletion?

Over the last two decades, several methods of TCD have been used to limit the risk of GVHD following AlloSCT. These included physical methods (e.g. soybean lectin agglutination plus E-rosette depletion), immunologic methods (e.g. monoclonal antibody plus complement), and combined physical/immunologic methods (e.g. immunomagnetic beads). Of these, immunomagnetic bead depletion using the CliniMACS device has been the most efficient and reproducible from center to center and is suitable for expanding to multiple centers in a subsequent Phase III trial. Furthermore, of the current techniques providing a level of T cell depletion sufficient to prevent both acute and chronic GVHD, the CliniMACS System presents several advantages. The single step positive selection of $CD34^+$ precursors from peripheral blood stem cells (PBSC) by the CliniMACS system provides the requisite level of T cell depletion required to prevent GVHD in both HLA-matched and HLA-disparate hosts. The CliniMACS system already has a registered master file at the Food and Drug Administration (FDA), and several Investigational Device Exemptions (IDEs) have already been granted for trials using the CliniMACS system. An IDE has been obtained from the FDA (BB-IDE #11965) on the basis of this protocol (#0303). Finally, many BMT CTN centers already have the CliniMACS device and are familiar with its operation.

While clinical experience with the use of CliniMACS-separated $CD34^+$ PBSC for HLA-matched transplants for AML in early remission is limited, the doses of $CD34^+$ cells and $CD3^+$ T cells provided by these grafts is extensively documented. Furthermore, with a cytoreductive regimen including TBI/thiotepa/ATG and either cyclophosphamide or fludarabine, engraftment and durable hematopoietic reconstitution have been achieved in 100% of HLA-matched transplants (N=56), and 95-100% of HLA-disparate transplants (N=140) using grafts processed with the CliniMACS system. Grades II-IV acute GVHD rates range from 0-4% for patients transplanted with CliniMACS $CD34^+$ PBSC grafts without further GHVD prophylaxis. Relapse rates for AML patients transplanted in remission were 11-13%. Thus, this method of TCD via $CD34^+$ cell selection was considered the best choice for further study in the multi-center Phase II trial.

5. How were the minimal and optimal $CD34^+$ and $CD3^+$ cell doses chosen?

Studies performed in recipients of T cell depleted AlloSCT at MSKCC, Perugia, and elsewhere strongly suggest that in order to reliably prevent the development of moderate to severe acute GVHD AlloSCT, $CD3^+$ cell doses $< 1.0 \times 10^5$/kg recipient weight must be maintained. Higher doses are associated with a greater risk of GVHD. This trial will use cytokine mobilized peripheral blood stem cells (PBSC) from HLA-identical donors in preference to bone marrow in order to increase the number of $CD34^+$ cells transplanted. However, this will also result in a one log increase in the number of $CD3^+$ cells collected prior to TCD. Nevertheless, data from several centers demonstrate that $CD34^+$ cell selection using the CliniMACS device consistently results in a 4-5 log reduction in $CD3^+$ cell dose. This level of TCD should ensure that allografts transplanted on this trial contain a $CD3^+$ cell dose $< 1.0 \times 10^5$/kg.

Less data are available to determine the $CD34^+$ cell dose required to ensure prompt, consistent, and sustained hematopoietic engraftment following T cell depleted transplantation. However, several trials have demonstrated that higher $CD34^+$ cell doses are associated with better overall outcomes following T cell replete AlloSCT, and $CD34^+$ cell dose is known to be an important variable influencing engraftment after T cell deplete transplants. A $CD34^+$ cell dose of 5.0×10^6/kg was selected as a target based both on prior Phase III studies of PBSC transplantation as well as practical issues related to the likely yield of $CD34^+$ cells following processing on the CliniMACS device. This is also a reasonable target based on the number of $CD34^+$ cells collected with 2 phereses and the 50-60% yield of $CD34^+$ cells following selection with the CliniMACS device. The minimum $CD34^+$ cell dose of 2.0×10^6/kg was based in part on data from T cell depleted bone marrow transplants and experience at participating centers demonstrating that $CD34^+$ doses greater than 2.0×10^6/kg typically result in prompt engraftment. Realizing the PBSC and bone marrow grafts are not equivalent, this lower limit was chosen somewhat empirically based on the data described above.

6. How were the study endpoints chosen?

Based on published results of unmodified transplants from HLA-matched siblings applied to patients with AML in CR1 or CR2, a significant improvement in results would be achieved if

DFS at 6 months was > 75%, the true incidence of transplant-related mortality at 1 year was < 30% and DFS rate at 2 years was $\geq$ 70% for patients transplanted in first remission and > 60% for patients transplanted in second remission. Additional endpoints include: proportion of grafts failing to meet the target $CD3^+$ and $CD34^+$ cell dose, graft failure rate, and incidences of acute grade II-IV and chronic GVHD.

The choice of disease-free survival (DFS) at 6 months as the primary endpoint represents a balance between what the protocol team thinks is a clinically meaningful result and the desire to reach a decision on moving forward with a Phase III trial as expeditiously as possible. If the Phase II data is sufficiently promising, based on a 6 month DFS estimate of > 75%, a Phase III trial against traditional GVHD prophylaxis is warranted. Death or relapse are events for this endpoint. If the strategy in the Phase II study is successful, a Phase III trial comparing T cell depleted PBSC transplants with unmanipulated PBSC and traditional GVHD prophylaxis will be designed.

7. Is the accrual goal of up to 45 patients in one year feasible?

We carefully reviewed the CIBMTR/ABMTR database to determine the number of transplants for AML in CR1 or CR2 reported to the registry on an annual basis. We next looked at the transplant activity at the individual centers included in this multi-center trial to determine the number of HLA-identical transplants for AML performed each year. It appeared that each of the 8 centers could contribute on average 5-6 patients to this trial over the course of a year with some centers expecting ~3 patients and others as many as 8 patients. This would result in a total of 40-48 patients available for accrual over the course of a year. With a goal to accrue 45 patients with an evaluable graft, this trial should be able to be completed within one year of activation.

8. Are there research blood samples collected during the trial?

During the trial, blood samples are required to monitor immune reconstitution and for EBV reactivation. These are both undertaken to improve the overall safety of the trial therapy. However, as these types of studies are not routinely performed as standard of care, they are being paid for by the BMT CTN and will not be billed to the patient and/or insurance provider. These are required observations/tests and are NOT optional. This is different from other BMT CTN protocols in which some samples are collected for future research and sent to a central repository.

9. Do all patients have to be registered with the CIBMTR?

The data collection system and monitoring system for BMT CTN trials builds on the CIBMTR data collection system. An essential part of this process is registration of ALL patients transplanted in each participating center. This requires completion of the CIBMTR "Pre-Reg Form". **Registration of transplant recipients (both those entered and those not entered on BMT CTN trials) is a requirement for all Core and non-Core centers.** These data will be

used to generate recruitment reports that would otherwise be requested of each center monthly to meet NHLBI recruitment monitoring requirements. They will also be used to study the recruitment process and evaluate ways in which we can modify protocols or institute other measures to enhance accrual to BMT CTN protocols.

What forms must I submit to the CIBMTR?

- ✓ **Pre-Registration Form** for ALL patients **(**both those entered and those not entered on BMT CTN trials) is a requirement for all Core and non-Core centers. At a minimum these must be batched to the CIBMTR **monthly.**
- ✓ **TED Form** (including the Core, Graft and Disease inserts) at 100 days post transplant for all patients enrolled in BMT CTN protocols. At a minimum these must be batched quarterly to the CIBMTR.
- ✓ **Follow-up TED Form** (including the Core and Disease follow-up inserts) yearly for all patients enrolled in BMT CTN protocols. At a minimum these must be batched quarterly to the CIBMTR.

How do I submit the CIBMTR forms?

You may submit "paper" or "electronic" CIBMTR forms directly to the CIBMTR as you currently do. If you chose to submit paper forms, green "BMT CTN" stickers will be provided to you to place on the forms to notify the CIBMTR that the patient is participating in a BMT CTN protocol(s).