PROTOCOL SYNOPSIS

A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus Host-Disease

Co-Principal Investigators: Leo Luznik, Miguel-Angel Perales, Marcelo Pasquini

Study Design: The study is designed as a three arm randomized Phase III, multicenter trial comparing two calcineurin inhibitor (CNI)-free strategies for GVHD prophylaxis to standard calcineurin inhibitor tacrolimus and methotrexate (Tac/Mtx) in patients with acute leukemia or myelodysplasia undergoing myeloablative conditioning hematopoietic stem cell transplantation.

Primary Objective: The primary objective of the randomized trial is to compare chronic GVHD/relapse-free survival [CRFS] as a time to event endpoint after hematopoietic stem cell transplant (HSCT) between each of the CNI-free interventions and a Tac/Mtx control.

Secondary Objectives: Secondary objectives are: comparison of rates of grade II-IV and III-IV acute GVHD, chronic GVHD, chronic GVHD-free survival, immunosuppression-free survival at one year, primary and secondary graft failure, neutrophil and platelet engraftment, disease relapse, transplant-related mortality, rates of Grade ≥ 3 toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0; incidence of CMV and EBV reactivation, incidence of infections; immune reconstitution, quality of life and overall survival.

Eligibility Criteria: Patients ≥ 1 year and < 66 years undergoing HSCT for treatment of acute leukemia in morphologic complete remission with or without hematologic recovery or myelodysplasia with < 5% blasts in the marrow and no circulating blasts, and who are eligible for a myeloablative allogeneic transplant. Patients with CMML must have a WBC count ≤ 10,000 cells/μL and < 5% blasts in the marrow. Patients with ≥ 5% blasts due to a regenerating marrow must contact the protocol chairs for review. Patients must have a related or unrelated donor. Related donor must be an 8/8 match for HLA-A, -B and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Pediatric related donors must weigh ≥ 25.0 kg, must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter, must be willing to (1) donate bone marrow and (2) receive G-CSF

	followed by donation of peripheral blood stem cells (product to be determined by randomization post enrollment), and must meet institutional criteria for donation. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria. At time of enrollment, the donor should not have any known preferences or contraindications to donate bone marrow or peripheral blood stem cells.
Treatment Description:	Patients will be randomized to receive one of the three specified interventions: 1) CD34 selected T-cell depleted peripheral blood stem cell (PBSC) graft; 2) unmanipulated bone marrow (BM) graft followed by cyclophosphamide (Cy) 50mg/kg Day +3 and +4 post HSCT; or, 3) unmanipulated BM graft with Tac/Mtx GVHD prophylaxis. Tac will be maintained at therapeutic doses for a minimum of 90 days. Methotrexate will be dosed at 10-15mg/m^2 for a maximum of 4 doses post-transplant.
Accrual Objective:	The clinical trial will enroll 345 patients or 115 per arm, in an adaptive design for futility evaluated at time of interim analysis.
Accrual Period:	The estimated accrual period is 42 months.
Study Duration:	Patients will be followed for 2 years following hematopoietic cell transplantation.
Interim Analysis:	No formal interim analyses for efficacy will be used. There is also not included in the design an option for closure of the control group while keeping the two treatment arms open, in the event that at least one of the treatments demonstrate early efficacy. Interim analyses for futility will be conducted at times coincident with regularly scheduled meetings of the DSMB, starting when approximately 45-50% of the targeted number of events have been observed.
Stopping Guidelines:	Monitoring of a key safety endpoint (mortality) will be conducted monthly up to 100 days post-randomization separately in each of the three treatment arms. At least three events must be observed in order to trigger review.
Correlative Studies:	Comparison of immune reconstitution using a panel of clinically available tests across all treatment arms. Advanced immune reconstitution assays.

STUDY SCHEMA

Aim: To determine if either CNI-free approach improves the rate of chronic GVHD and relapse free survival after transplant compared to standard Tac/Mtx control.

Inclusion Criteria

1. Males and females aged ≥ 1.0 year and < 66.0 years
2. Patients with acute leukemia, in morphologic complete remission with or without hematologic recovery or myelodysplasia with < 5% blasts in the marrow and no circulating blasts. Patients with CMML must have a WBC count ≤10,000 cells/μL and < 5% blasts in the marrow. Patients with ≥ 5% blasts due to a regenerating marrow must contact the protocol chairs for review.
3. Planned myeloablative conditioning regimen (see eligible regimens in Table 2.4).
4. Patients must have a related or unrelated donor as follows:
   a. Related donor must be an 8/8 match for HLA-A, -B and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Pediatric related donors must weigh ≥ 25.0 kg, must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter, must be willing to (1) donate bone marrow and (2) receive G-CSF followed by donation of peripheral blood stem cells (product to be determined by randomization post enrollment), and must meet institutional criteria for donation.
   b. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria. At time of enrollment, the donor should not have any known preferences or contraindications to donate bone marrow or peripheral blood stem cells.
      i. Selection of unrelated donors is to be performed according to institutional practice. It is recommended that the time from collection to initiation of the cell processing be considered when prioritizing donors as data shows better results for CD34 selection when processing is started within 36 hours of the end of collection as indicated in section 2.5.1.2.
5. Cardiac function: Ejection fraction at rest ≥ 45% or shortening fraction of ≥ 27% by echocardiogram or radionuclide scan (MUGA**).**
6. Estimated creatinine clearance (for patients > 12 years) greater than 50 mL/minute (using the Cockcroft-Gault

Exclusion Criteria

1. Prior autologous or allogeneic hematopoietic stem cell transplant
2. Karnofsky or Lansky Performance Score < 70%
3. Active CNS involvement by malignant cells
4. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment
5. Presence of fluid collection (ascites, pleural or pericardial effusion) that interferes with methotrexate clearance or makes methotrexate use contraindicated
6. Patients seropositive for HIV-1 or -2
7. Patients seropositive for HTLV-I or –II
8. Patients with active Hepatitis B or C viral replication by PCR
9. Documented allergy to iron dextran or murine proteins
10. Women who are pregnant (positive serum or urine βHCG) or breastfeeding
11. Females of childbearing potential (FCBP) or men who have sexual contact with FCBP unwilling to use 2 effective forms of birth control or abstinence for one year after transplantation
12. History of uncontrolled autoimmune disease or on active treatment
13. Patient with prior malignancies, except resected non-melanoma or treated cervical carcinoma in situ. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.
14. Patient unable to comply with the treatment protocol, including appropriate supportive care, follow-up and research tests
15. Planned post-transplant maintenance therapy except for FLT3 inhibitors or TKIs
    a. Must be declared prior to randomization

formula and actual body weight); for pediatric patients (≥ 1 year to 12 years), GFR estimated by the updated Schwartz formula ≥ 90 ml/min/1.73 m^2. If the estimated creatinine clearance is < 90 ml/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 70 mL/minute/1.73m^2. 7. Pulmonary function: DLCO ≥50% (adjusted for hemoglobin), and FVC and FEV1≥50%; for children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea and no need for supplemental oxygen as evidenced by O_2 saturation ≥ 92.0% on room air. 8. Liver function: total bilirubin < 2x the upper limit of normal (unless elevated bilirubin is attributed to Gilbert's Syndrome) and ALT/AST < 2.5x the upper normal limit. 9. Signed informed consent	16. Planned use of cryopreserved hematopoietic stem cells 17. *German centers only:* Treatment with any known non-marketed drug substance or experimental therapy within 5 terminal half lives or 4 weeks prior to enrollment, whichever is longer, or participation in any other interventional clinical study.

Primary endpoint:
- Chronic GVHD/relapse-free survival (CRFS): this time to event outcome is defined as moderate to severe chronic GVHD by the NIH consensus criteria, disease relapse, or death by any cause.

Secondary endpoints:
- Grades II-IV and III-IV acute GVHD
- Chronic GVHD
- Chronic GVHD-free survival
- Immunosuppression-free survival at 1 year
- Hematologic recovery
- Primary and secondary graft failure
- Immune reconstitution
- Disease relapse
- Transplant-related mortality
- Toxicity (SOS and IPS) and rates of infections (CMV and EBV reactivation)
- Relapse-Free and overall survival
- Quality of life

Allowed Myeloablative Conditioning Regimens		
CD 34 Selected Arm	PTCy Arm	Control Arm
Cy/TBI/Thiotepa/ ATG Bu/Mel/Flu/ATG	Cy/TBI Flu/Bu Bu/Cy	Cy/TBI TBI/Etoposide Flu/Bu Bu/Cy

***Busulfan dose >8mg/kg or IV equivalent**

***Immunosuppression Taper*:**
Patients without GVHD
Tacrolimus
- Taper to initiate at least by Day 90 and completely off at Day 180.

Outline of Treatment Plan