Title

Efficacy evaluation of post-transplant cyclophosphamide-based graft-versus-host disease prophylaxis with ATG, calcineurin inhibitor-free, for matched-sibling or matched-unrelated transplantation

Study protocol and statistical analysis plan

Version 1.00

Short title

PTCy and ATG for MSD and MUD transplants

Authors

Leonardo Javier Arcuri

Simone Pereira Lermontov

Natalia Laso

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Brief summary

Hematopoietic stem cell transplantation is a curative treatment for a number of benign and malignant hematologic diseases. One of the key parts of hematopoietic stem cell transplantation is the prophylaxis of graft-versus-host disease. Since the end of the 1970s, with the introduction of cyclosporine, calcineurin inhibitors (cyclosporine and tacrolimus) have become part of almost all prophylactic regimens, even though they are a group of drugs with a poor toxicity profile that requires monitoring. constant serum level. Since 2008, post-transplant cyclophosphamide has been introduced with great success, associated with a calcineurin inhibitor and mycophenolate, in the prophylaxis of graft-versus-host disease in haploidentical transplantation (50% matched).

Since then, in view of this enormous success, efforts have been made to incorporate post-transplant cyclophosphamide in matched related and unrelated transplants, or with a mismatch.

This is a prospective, 2-arm, non-randomized study. Arm 1, with related donors, and arm 2, with unrelated donors. Patients will be allocated in these arms according to donor availability (patients with a matched-sibling donor will receive a matched-sibling transplant; patients with no related donors but with unrelated donors, an unrelated transplant).

Patients who are ready for transplantation with matched-sibling or unrelated donors will be recruited to participate in the study.

The stem cell collection target will be 5E6 CD34/kg recipient weight for peripheral source. If a quantity greater than this is collected, the remainder will be cryopreserved according to the institutional protocol.

Graft-versus-host disease prophylaxis will be performed on D+3 and D+4 with cyclophosphamide and with ATG on D-1 or on D-2 and D-1, depending on ATG de-escalation, for matched-sibling transplants, according to prespecified criteria based on the 3+3 approach; and on D+3 and D+4 with cyclophosphamide and with ATG on D-2 and D-1, for unrelated donors.

Introduction

Hematopoietic stem cell transplantation is a curative treatment for a number of benign and malignant hematologic diseases[1]. One of the key parts of hematopoietic stem cell transplantation is the prophylaxis of graft-versus-host disease. Since the end of the 1970s, with

the introduction of cyclosporine, calcineurin inhibitors (cyclosporine and tacrolimus) have become part of almost all prophylactic regimens, even though they are a group of drugs with a poor toxicity profile that requires monitoring. constant serum level. Since 2008, post-transplant cyclophosphamide has been introduced with great success, associated with a calcineurin inhibitor and mycophenolate, in the prophylaxis of graft-versus-host disease in haploidentical transplantation (50% matched)[2].

Since then, in view of this enormous success, efforts have been made to incorporate post-transplant cyclophosphamide in matched related and unrelated transplants, or with a mismatch. The randomized trial BMT CTN 1301[3] showed that when the source is bone marrow and the donor is 100% compatible, the use of post-transplant cyclophosphamide as a single agent is possible and may be the new gold standard of prophylaxis.

The randomized study BMT CTN 1703[4] showed that, when the source is peripheral blood and the donor is 100% compatible, the combination of post-transplant cyclophosphamide with a calcineurin inhibitor and mycophenolate offers the best results. Kunacheewa et al[5] has shown the viability of calcineurin-free prophylaxis, with ATG 5.0 mg/kg and post-transplant cyclophosphamide, when the source is peripheral and the donor is fully compatible.

Taken together, these results show that post-transplant cyclophosphamide-based prophylaxis, free of calcineurin inhibitor, is possible and offers excellent results both with bone marrow and peripheral blood sources.

Justification

Results of prophylaxis based on cyclophosphamide after transplantation with ATG, free of calcineurin inhibitors, are available in the international literature, but they are not available in Brazil, where there is a highly miscegenated population. Knowing these results in Brazil would be of great importance.

Currently, the ATG dose of 5 mg/kg, associated with cyclophosphamide post-transplantation, for HLA-compatible transplantation, is considered safe based on a previous phase II study, and this is the dose that we will use in unrelated transplantation. However, lower doses for related transplantation, which may be just as effective and less toxic, have not been tested. This study will help define the optimal dose of ATG for related transplantation.

Ciclosporin has an average cost of around BRL 1,100.00 per month while tacrolimus has a cost of around BRL 2,100.00 (source: br.kairosweb.com, accessed on 01/20/2023), and the patient usually uses the medication for 6 months. There is also the cost of dosing cyclosporine or

tacrolimus, which is done twice a week while the patient is using the medication (cost: BRL 335.00 for each dosage of cyclosporine and BRL 460.00 for each dosage of tacrolimus; Fleury Laboratory, price carried out on 01/20/2023). This generates a potential cost of around BRL 24,000.00 per patient for cyclosporine and BRL 37,000.00 for tacrolimus, per patient. Furthermore, as described earlier, calcineurin inhibitors (cyclosporine and tacrolimus) have a poor toxicity profile. Demonstrating the feasibility of a calcineurin inhibitor-free protocol could help reduce costs and improve patients' quality of life. This study will have the differential of collecting quality of life.

Additionally, since 2021, the availability of calcineurin inhibitors and the dosage of these drugs has been intermittent at Instituto Nacional de Cancer (INCA). Demonstrating the feasibility of a calcineurin inhibitor-free protocol could prevent transplants from being suspended when calcineurin inhibitors or their dosage kit are not available.

Objectives

General

To evaluate the efficacy of post-transplant cyclophosphamide-based graft-versus-host disease prophylaxis with ATG, free of calcineurin inhibitor, for related or unrelated transplantation

Specific

Identify the ideal dose of ATG in matched-sibling transplant, peripheral graft, when combined with post-transplant cyclophosphamide.

Describe overall and relapse-free survival;

To describe the incidences of acute grade II-IV, III-IV, corticosteroid-refractory, chronic, and chronic corticosteroid-requiring graft-versus-host disease;

Describe the rate of relapse and non-relapse mortality;

To describe the incidences of CMV reactivation, CMV disease, and post-transplant lymphoproliferative disease;

Describe the quality of life after transplantation;

Describe CD4 and CD8 recovery kinetics.

Hypothesis

The incidence of grade III-IV graft-versus-host disease with cyclophosphamide after ATG transplantation alone is low.

Methods

It is a prospective, 2-arm, non-randomized study. Arm 1, with related donors, and arm 2, with unrelated donors. Patients will be allocated in these arms according to donor availability (patients with a matched-sibling donor will receive a matched-sibling transplant; patients with no related donors but with unrelated donors, an unrelated transplant).

Inclusion criteria

- Patient with (1) acute leukemia in first or second remission; (2) myelodysplasia with less than 20% blasts; (3) Hodgkin's or non-Hodgkin's lymphoma, in partial remission after salvage therapy

- Who will receive a related or unrelated, HLA-compatible transplant;

- Who is a transplant candidate with FluMel, FluTBI, CyTBI, BuCy or BuFlu conditioning;

- Peripheral blood source;

- Age between 18 and 60 years.

Exclusion criteria

- Renal dysfunction (Cr > 1.5 mg/dL)

- Hepatic dysfunction (transaminases x2 the normal value)

Study design

Patients who are ready for transplantation with matched-sibling or unrelated donors will be recruited to participate in the study. There will be no prioritization of research participants in the transplant queue. The consent form will be applied before the start of the conditioning for the transplant, when the inclusion and exclusion criteria will be reviewed.

The stem cell collection target will be 5E6 CD34/kg recipient weight for peripheral source. If a quantity greater than this is collected, the remainder will be cryopreserved according to the institutional protocol.

Graft-versus-host disease prophylaxis will be performed on D+3 and D+4 with cyclophosphamide and with ATG on D-1 or on D-2 and D-1, depending on ATG de-escalation, for matched-sibling transplants, according to the following section; and on D+3 and D+4 with cyclophosphamide and with ATG on D-2 and D-1, for unrelated donors.

Outcomes will be reported according to the research team's assessment as per the section "Definition of Outcomes", i.e., there will be no external event adjudication committee.

In case of graft-versus-host disease grades II-IV, classified according to the MAGIC[6] criteria, patients will be treated with corticosteroid therapy equivalent to methylprednisolone 2 mg/kg for up to 2 weeks (which may be shortened in case of complete response prior to two weeks), and tapered according to the scheme in Table 1. The prednisone dose is 1.25x higher than the methylprednisolone dose. At the discretion of the medical team treating the patient, cyclosporine or tacrolimus may be associated with steroid therapy.

Table 1. Corticosteroid tapering

Semana	**Methylprednisolone equivalent**
1st week	2 mg/kg div. 12/12h
2nd week	2 mg/kg 1x/day
3rd week	1 mg/kg 1x/day
4th week	0.5 mg/kg 1x/day
5th week	0.4 mg/kg 1x/day
6th week	0.3 mg/kg 1x/day
7th week	0.2 mg/kg 1x/day
8th week	0.1 mg/kg 1x/day
9th week	0.1 mg/kg every other day
10th week	Discontinue

In case of chronic graft-versus-host disease requiring corticosteroids, the dose and duration of treatment will be in accordance with the medical team responsible for monitoring and treating the patient.

In cases of refractoriness to corticosteroids, whether in the acute or chronic form, treatment will be based on the best available supportive therapy, to be decided and applied by the medical team treating the patient.

The patient's follow-up will be carried out on the dates of regular consultations in the immediate post-discharge period, D+100, D+180 and annually. The exams performed will be

the same as for routine consultations. The vaccination program will be carried out according to the CEMO routine, starting 3 months after the transplant.

Treatment design

Patients who are scheduled to receive one of the following conditions will be eligible to enter the study:

FluMel

- Fludarabine 40 mg/m2 on days D-6, D-5, D-4 e D-3

- Melfalan 140 mg/m2 on day D-1

BuFlu

- Busulfan 3.2 mg/kg on days D-6, D-5, D-4 e D-3

- Fludarabin 40 mg/m2 on days D-6, D-5, D-4 e D-3

BuCy

- Busulfan 3.2 mg/kg on days D-7, D-6, D-5 e D-4

- Cyclophosphamide 50 mg/kg on days D-3 e D-2

FluTBI

- Fludarabine 40 mg/m2 on D-6, D-5 e D-4

- Total body irradiation 200 cGy 12/12h on D-3, D-2 e D-1

CyTBI

- Cyclophosphamide 50 mg/m2 on D-6 e D-5

- Total body irradiation 200 cGy 12/12h on D-3, D-2 e D-1

Graft-versus-host disease prophylaxis will be carried out with post-transplant cyclophosphamide:

- Cyclophosphamide 50 mg/kg on D+3 and D+4

Patients will receive MESNA associated with cyclophosphamide according to the institutional protocol; patients who receive busulfan will receive anticonvulsants according to the institutional protocol.

Additionally, patients will receive ATG:

- HLA-matched related, peripheral blood:

ATG 2.5 mg/kg on D-1 ± 1.5 mg/kg on D-2 (according to the flowchart below)

(total dose 4.0 mg/kg or 2.5 mg/kg, according to the flowchart below)

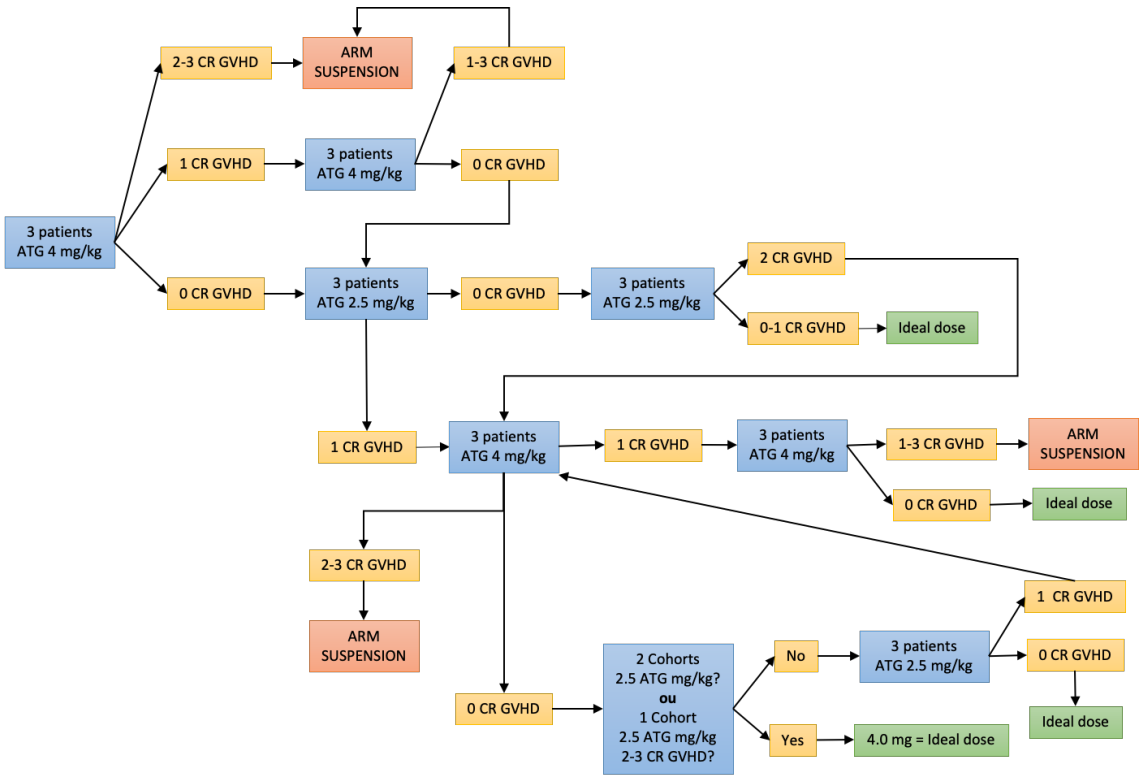
- Unrelated to peripheral blood

- ATG 2.5 mg/kg on D-2 and D-1

Patients receiving ATG will receive prophylaxis for drug reaction and serum sickness as per institutional protocol. They will receive antifungal prophylaxis with an azole agent active against Aspergillus sp.

The first 3 patients in arm 1 (related) will also receive ATG 2.5 mg/kg on D-1 and ATG 1.5 mg/kg on D-2, for a total dose of 4 mg/kg. The dose reduction and escalation scheme, according to the occurrence of corticorefractory graft-versus-host disease, is shown in the flowchart below, and aims to find the ideal dose for matched-sibling transplantation.

Fluxogram 1. De-escalation and dose escalation schedule



Legend: CR GVHD, corticorefractory graft-versus-host-disease.

Independent variables

The independent variables studied will be age, gender, disease, donor and recipient CMV, donor and recipient ABO typing, comorbidity index (HCT-CI[7]), disease status, disease risk index[8], pre-transplant minimal residual disease, donor type, donor sex, donor age, HLA match at 8 loci (A, B, C and DR) and number of infused CD34 cells.

Tabela 2. *Dummy table*

Variable	Cy+ATG2.5	Cy+ATG4.0	Cy+ATG5.0	Total
Median age (IQR)				
Gender				
Comorbidity index (HCT-CI) **1** **2** **3+**				
Disease **Acute lymphoblastic leukemia** **Acute myeloid leukemia** **Myelodysplastic syndrome** **Hodgkin lymphoma** **Non-Hodgkin lymphoma**				
MRD+ (for acute leukemias)				
Disease risk index **Low/intermediate** **High/very high**				
CMV receptor/donor **+/+** **+/-** **-/+** **-/-**				
ABO compatibility **Compatible** **Minor** **Major** **Bidirecional**				
Doador **MSD** **URD HLA 8x8**				
Graft **Peripheral blood**				
Conditioning **FluMel** **BuFlu** **BuCy** **FluTBI** **CyTBI**				
CD34 cells (mediana/IQR)				

Outcomes

The outcomes studied will be:

Death (overall survival);

Graft-versus-host disease (incidence) acute grades II-IV, acute grades III-IV, acute corticorefractory, chronic, and chronic requiring treatment with corticosteroids;

Relapse of malignant disease (incidence);

Mortality not related to relapse (incidence);

Composite death/relapse outcome (relapse-free survival);

CMV reactivation (incidence); CMV disease (incidence); EBV reactivation (incidence); post-transplant lymphoproliferative disease (incidence);

Quality of life after immediate discharge, D+100, D+180, D+360 and D+720; The FACT-BMT[9] (Functional Assessment of Cancer Therapy-Bone Marrow Transplant) is composed of six domains: physical well-being, socio-family well-being, relationship with the doctor, emotional well-being, functional well-being and additional concerns. The average time spent on the application is 15 minutes;

CD4 and CD8 at months 3, 6, 9 and 12.

Outcomes definition

Death – date of death

Relapse – presence of ≥ 5% blasts in bone marrow or ≥ 1% blasts in peripheral blood, confirmed by immunophenotyping, or presence of biopsy-confirmed extramedullary relapse

Non-relapse mortality – death in a patient with disease in remission

Acute graft-versus-host disease – graded according to the MAGIC criteria[6]

Corticorefractory acute graft-versus-host disease[10] – progression in any organ within 5 days after corticosteroid therapy equivalent to 2 mg/kg methylprednisolone, no response at 7 days, incomplete response at 28 days, or need to add some other immunosuppressant.

Chronic graft-versus-host disease – graded according to the 2014 NIH criteria[11]. They will also be classified according to the need for treatment with corticosteroids.

CMV reactivation – Two measurements with more than 100 copies in plasma, one measurement with more than 300 copies, or any measurement that led to preemptive treatment with ganciclovir

CMV disease – disease in the gastrointestinal tract confirmed by biopsy; lung disease characterized by clinical symptoms and radiological signs associated with reactivation of CMV in plasma or identification of CMV on bronchoscopy

Post-transplant lymphoproliferative disease – characterized by identification of EBV in plasma or whole blood associated with clinical or radiological symptoms, or biopsy of the affected region

Quality of life - measured by the FACT-BMT instrument, in months 3, 6, 12 and 24

CD4 and CD8 – measured at months 3, 6, 9 and 12, in cells/mm3

Statistical methods

The analyzes will be carried out by intention to treat, which means that patients who, for example, receive the treatment without having met all the exclusion criteria, but who have signed the consent form, will be included in the analysis.

Categorical variables will be presented as number and percentage, and continuous variables will be presented as median and interquartile range. Eventual comparisons between baseline characteristics of the population will be made using the chi-square or Fisher's exact test, as appropriate, for categorical variables, and the Mann-Whitney test for continuous variables.

Survival curves will be constructed using the Kaplan-Meier method and compared using the logrank method. Accumulated incidence curves will be constructed and compared using the Gray method. Univariate and multivariate analyzes will be performed using the Cox model. Multivariate Cox models will be constructed by the smallest AIC.

Sample size

Based on the number of transplants performed at the institution per year, we expect to recruit approximately 50 patients in 5 years (10/year).

Ethics

Patients will sign a consent form before being included in the study. This study will follow Resolution 466/12 and the Declaration of Helsinki.

Once approved by the local Ethics Committee, the protocol will be translated to English and registered at clinicaltrials.gov

Risks

There is a risk of loss of confidentiality. We will minimize this risk by collecting data anonymously. There is also the risk of graft-versus-host disease, but this risk is inherent to hematopoietic stem cell transplantation and previous studies have not shown an increase in the incidence of this complication with the prophylactic scheme used (on the contrary, they show a very low incidence).

Benefits

Because it is a study, the patient cannot expect any benefit. The main benefit of this research is to make it possible for patients who undergo bone marrow transplantation in the future to benefit from the results achieved in the current study.

Costs

Cyclophosphamide at a dose of 50 mg/kg for two days costs BRL 460.00 for an adult weighing 70 kg.

ATG at a dose of 2.5 mg/kg has a cost of BRL 4,860.00 per patient weighing 70 kg.

The estimated cost, over 5 years and per 50 patients, is detailed in the table below.

Tabela 3. Custo detalhado

Item	Cost per patient	Number of patients	Cost
Cyclophosphamide	R$ 460,00	50	R$ 23,000.00

Item	Cost per patient	Number of patients	Cost
ATG 2.5 mg/kg	R$ 4.860,00	25	R$ 121,500.00
ATG 5.0 mg/kg	R$ 9.720,00	25	R$243,000.00
Total		**25 in Cy+ATG2.5 group** **25 in Cy+ATG5.0 group**	**R$ 387,500.00**

It should be noted that the ATG dose used in the service is 4 mg/kg for related transplants from peripheral blood sources and 6 mg/kg for non-related transplants, any source. The service also uses cyclosporine or tacrolimus associated with methotrexate to prevent graft-versus-host disease, requiring monitoring of serum levels of cyclosporine or tacrolimus, whose costs have already been discussed in the "Justification" section. Therefore, there will be no increase in expenses, on the contrary, there will be a reduction in service expenses.

There will be no external funding.

Timeline

Table 4. Timeline

Action	Dates (DD/MM/YYYY)
Submission to Ethics Committee	07/03/2023
Team training	01/05/2023 – 31/05/2023
Patients inclusion	01/06/2023 – 01/06/2028
Partial results analyses	01/06/2025 – 30/06/2025
Submission for meeting	01/07/2025 – 31/07/2025
Analyses of the results	01/06/2030 – 30/06/2030
Preparation for submission	01/07/2030 – 31/07/2030
Submission for publication	01/08/2030 – 31/08/2030

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