

## ORIGINAL ARTICLE

# Determine safety of outpatient chemotherapy and autotransplants using refrigerated, non-frozen grafts in persons with multiple sclerosis

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## Abstract

**Background:** Persons with multiple sclerosis are increasingly treated with intermediate- or high-dose chemotherapy and a hematopoietic cell autotransplant. This is often done in an inpatient setting using frozen blood cell grafts.

**Objective:** Determine if chemotherapy and a hematopoietic cell autotransplant can be safely done in an outpatient setting using refrigerated, non-frozen grafts.

**Methods:** We developed an autotransplant protocol actionable in an outpatient setting using a refrigerated, non-frozen blood graft collected after giving cyclophosphamide, 50 mg/kg/d × 2 days and filgrastim, 10 µg/kg/d. A second identical course was given 9 days later followed by infusion of blood cells stored at 4°C for 1-4 days. The co-primary outcomes were rates of granulocyte and platelet recovery and therapy-related mortality.

**Results:** We treated 426 consecutive subjects. Median age was 47 years (range, 21-68 years). A total of 145 (34%) were male. Median graft refrigeration time was 1 day (range, 1-4 days). Median interval to granulocytes >0.5 × 10E + 9/L was 8 days (range, 2-12) and to platelets >20 × 10E + 9/L, 8 days (range, 1-12). Only 15 subjects (4%) were hospitalized, predominately for iatrogenic pneumothorax (N = 5) and neutropenic fever (N = 4). There was only 1 early death from infection.

**Conclusion:** Intermediate-dose chemotherapy and a hematopoietic cell autotransplant can be safely done in an outpatient setting using, refrigerated, non-frozen grafts.

## KEYWORDS

Autotransplants, cyclophosphamide, multiple sclerosis, rituximab

## 1 | INTRODUCTION

Persons with multiple sclerosis (MS) are sometimes treated with intermediate- or high-dose chemotherapy and an autotransplant.<sup>1,2</sup> More than 1000 autotransplants are reported with MS<sup>2-6</sup> but use of autotransplants may be substantially more common as many cases are not reported. Recent data from a small randomized trial of less-intensive chemotherapy reported better outcomes with this approach compared with disease-modifying therapy in persons with advanced relapsing-remitting MS.<sup>7</sup> As such, autotransplant frequency is likely to increase.

As currently done autotransplants for MS are expensive and technically demanding requiring hospitalization and programmed freezing of the graft. We previously reported autotransplants for several hematologic neoplasms can be done in an outpatient setting using refrigerated, non-frozen blood cells.<sup>2,6</sup> We wondered whether the same modifications could be used in persons with MS? We report data from 426 subjects with MS receiving autotransplants as outpatients setting using refrigerated, non-frozen grafts.

### 1.1 | Subjects

We studied consecutive subjects with MS referred to our center starting June, 2015. Subject should not have received bone marrow toxic or immune suppressive drugs within the prior 6 months and have normal heart, liver, lung, and kidney. All subjects participated a study registered in *ClinicalTrials.gov* identifier NCT02674217 which was approved by the Ethics Committee of the Clinica RUIZ (*Conbioetica* 21CEI00120130605, Registry N. 13 CEI 21 114 126). Subjects gave written-informed consent. This report concerns only safety of the procedure.

### 1.2 | Blood cell collection

Blood cells were mobilized by cyclophosphamide, 50 mg/kg, given intravenously over 2 hours on d -11 and -10. Filgrastim, 10 µg/kg, was given twice daily on d -9 to -1 subcutaneously. Apheresis was done on d -2 using an Amicus® device (Fresenius Kabi) or a Spectra Optia® device (Terumo BCT) using the Spin-Nebraska® protocol<sup>8</sup> either by vein or via a Mahurkar®-type subclavian catheter. The objective was to collect  $\geq 1.0 \times 10^6$  viable CD34-positive cells/kg.

### 1.3 | Conditioning and infusion

Subjects were outpatients. Cyclophosphamide, 50 mg/kg intravenously, was given over 2 hours on d -11 and -10 followed by 2-mercaptoethane sulfonate Na (mesna), 1 g/mE + 2 over 3 hours. Treatment was repeated on d -2 and -1. All subjects received ondansetron, 8 mg, dexamethasone, 4 mg, and pantoprazole. Filgrastim (10 µg/kg, once daily), cotrimoxazole, fluconazole, and acyclovir were given until granulocytes  $>0.5 \times 10^6 + 9/L$  (Figure 1). Some subjects received rituximab posttransplant but not until after bone marrow recovery was assessed.

### 1.4 | Graft storage

The apheresis product and 1 mL aliquots thereof were kept in ACD-A (Baxter Healthcare) at 4°C in 1 L transfer packs (Baxter Healthcare) composed of gas impermeable, polyvinyl chloride plastic film for up to 96 hours. Enumeration of mononuclear (MNC) and CD34-positive cells was done by flow-cytometry in an EPICS Gallios® device (Coulter Electronics), using phycoerythrin labeled anti-CD34 HPCA-2 monoclonal antibody (Becton Dickinson) and a fluorescence isothiocyanate tagged anti-CD45 monoclonal antibody (Beckman Coulter), gating on 7' amino-actinomycin-D-excluding cells. Viability studies of the stored apheresis product used propidium iodide exclusion evaluated on a flow cytometer. The apheresis product was obtained on d -2 was infused on d 0 after storing in a conventional blood bank refrigerator (Thermoforma). In cases requiring  $>1$  apheresis, cells were infused 1 days after the last dose of cyclophosphamide.

### 1.5 | Statistics

This was a safety study with primary co-endpoints of granulocyte and platelet recoveries and therapy-related mortality (TRM). Efficacy was not assessed. Granulocyte recovery was defined as the interval from d 0 to granulocytes  $>0.5 \times 10^6 + 9/L$  for three consecutive d. Platelet recovery was defined as interval from d 0 to platelets  $>20 \times 10^9 + 9/L$  on three consecutive d without platelet transfusions. Data are presented as median and range. Therapy-related mortality was defined as death from any cause before d 100.

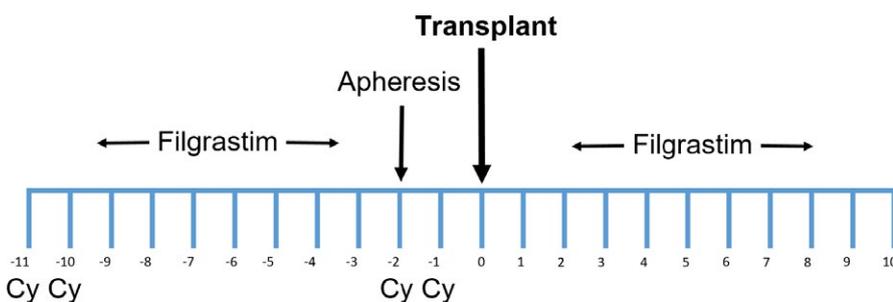


FIGURE 1 Transplant scheme

## 2 | RESULTS

### 2.1 | Subjects

A total of 426 subjects were enrolled after June, 2015. A total of 145 were male (34%). Median age was 47 years (range, 21-68 years).

### 2.2 | Apheresis

Subjects had a median of 1 apheresis (range, 1-4) with only 72 (17%) requiring >1 apheresis. A median of  $4.06 \times 10^6 + 6/\text{kg}$  viable CD34-positive cells was infused (range,  $1-32 \times 10^6 + 6/\text{kg}$ ).

### 2.3 | Hematopoietic recovery and TRM

Median intervals to granulocytes  $>0.5 \times 10^9/\text{L}$  and to platelets  $>20 \times 10^9/\text{L}$  were 8 days (ranges 2-12 and 1-12 days). A total of five subjects received RBC transfusions and two platelet transfusions. A total of 15 subjects were hospitalized within 28 days of their autotransplant with durations typically <2 days. Reasons for hospitalization included iatrogenic pneumothorax (N = 5), neutropenic fever (N = 4), MS flare (N = 2), nausea and/or vomiting (N = 2), cardiac arrhythmia (N = 1), and urinary tract infection (N = 1). There was no hemorrhagic cystitis. The only death within the first 100 days was from *Aeromonas veronii*.

## 3 | DISCUSSION

Our data indicate fractionated, high-dose cyclophosphamide and an autotransplant of a refrigerated, non-frozen graft can be safely in subjects with MS in an outpatient setting with rapid bone marrow recovery and only 1 therapy-related death. Rates of granulocyte and platelet recovery are like those reported using frozen grafts.<sup>9-12</sup> These modifications substantially decrease the costs and increase availability of this procedure in persons with MS, especially those in developing countries. Average cost of the procedure at our center was \$40 000 USD (range, \$35 000-45 000 USD). This compares with reported ranges of inpatient autotransplants for MS using frozen grafts of \$700 000-200 000 USD in other clinics in México (personal communication) and \$80 000-300 000 USD in the United States and EU.<sup>13</sup>

We collected data on numbers of CD34-positive cells but no further data on graft composition. This was intentional and based on our experience in other studies in which all subjects receiving  $\geq 1.0 \times 10^6 + 6$  CD34-positive cells/kg had rapid recovery of bone marrow function rapidly. This is an important because detailed characterization of different types of cells in the graft other than CD34-positive cells is complex, time-consuming, expensive, and provides no additional clinically usual data. Requiring more detailed studies of graft composition may limit applicability of autotransplants in developing countries.

An unusual aspect of our study was giving two 2 days blocks of cyclophosphamide 6 days apart rather than 4 days continuously.

This was done for three reasons: (a) use of cyclophosphamide to mobilize blood cells for the autograft; (b) allow using a refrigerated rather than a frozen autograft; and (c) decrease toxicity.<sup>14</sup> Our finding of rapid bone marrow recovery using refrigerated blood cell grafts is like our experience in persons with plasma cell myeloma and lymphomas receiving high-dose therapy and an autotransplant.<sup>15-17</sup>

Because this was a safety rather than an efficacy study, there are limitations to interpreting our data. For example, it was not designed to compare fractionated vs continuous cyclophosphamide nor refrigerated vs frozen grafts. In our setting and others, it is unknown whether an autograft is needed to accelerate bone marrow recovery, a question unlikely to be studied in a randomized trial. We do not report data on graft composition other than whether  $\geq 1.0 \times 10^6 + 6$  viable CD34-positive cells/kg were collected. Because we do not report efficacy data, we make no claim how our procedure, although simple and cost-effective, compares with other autotransplant approaches to treating MS.

In summary, we report a method to do autotransplants in persons with MS in an outpatient setting using refrigerated, non-frozen grafts. This approach is safe, reduces costs and increase availability of autotransplants for MS in developing countries.

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## CONFLICT OF INTEREST

RPG is a part-time employee of Celgene Corp.

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