

Processing of hematopoietic stem cells used for autologous transplantation, three years experience in Iceland.

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Introduction

High dose chemotherapy with autologous hematopoietic stem cell support has been used since the early 1980's to treat patients with different malignant diseases. The stem cells are harvested either from bone marrow or peripheral blood (after mobilization with G-CSF) usually during the patients second remission phase (Figure 1).

In December 2003 we started an autologous hematopoietic stem cell transplantation program at the National University Hospital in Reykjavík, Iceland

Here we summarize results from the cell processing part of the program for the first three years.

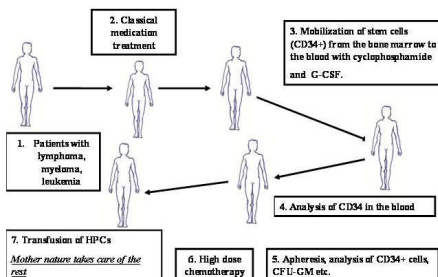


Figure 1. High dose chemotherapy followed by autologous stem cell support.

Methods

After treating the patients with cytotoxic drugs, mobilization of the stem cells from the bone marrow to the blood was induced with G-CSF. The collection (harvesting) of the stem cells was done using an apheresis machine (Amicus).

Phenotypic identification and enumeration of the cells was done by flow cytometry (FACSCalibur) using the following conjugated antibodies; CD45 for the total number of leukocytes and CD34 for hematopoietic stem/progenitor cells. Cell viability was determined by acridine-orange/ethium bromide staining (AO/Ebr).

After depleting the cells of the patient's plasma, they were re-suspended in donor AB plasma containing 10% DMSO, frozen in a controlled rate freezer and stored in liquid nitrogen.

After quick thawing of the cells, DMSO was removed and quality of the graft assessed for viability (AO/Ebr), cell number and function (CFU assay). The vital dye 7-amino actinomycin D (7-AAD) was added for more exact determination of viable CD34+ cells. For enumeration of CD34+ cells, the ISHAGE guidelines were used (dual platform).

Results

Figure 2 shows the number of autologous and allogeneic hematopoietic stem cell transplants in Icelandic patients in the period 1981 to 2006.

In the period under review successful mobilization was achieved in 45 out of 48 patients (Table I and II). Clinical characteristics of the patients are shown in Table II.

The total number of CD34+ cell analysis and apheresis procedures were 234 and 113 respectively.

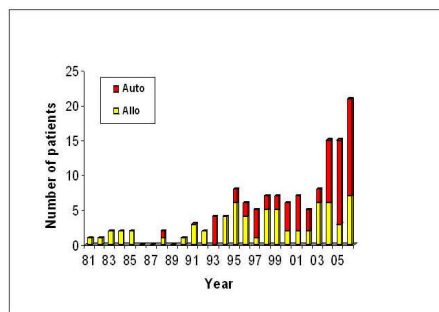
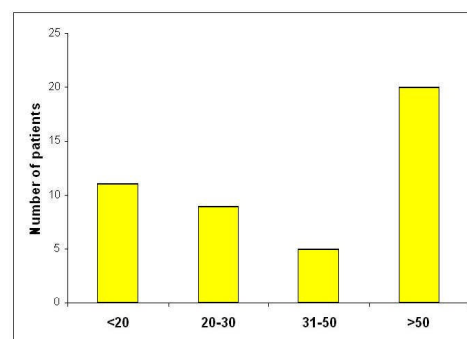


Figure 2. Number of hematopoietic stem cell transplants in Icelandic patients 1981-2006.

Table II shows the total number of viable CD34+ cells/kg, harvested and numbers re-infused, treatment. The mean percentage of post thaw recovery of CD34+ cells was 67,5%

Figure 4 shows the correlation between engraftment time and numbers of re-infused cells after high dose chemotherapy.



No. of apheresis ^{a)} :	3,5(2-5)	3 (2-4)	2,3 (2-3)	1,4 (1-2)
CD34x10 ⁶ b):	358 (216-587)	458 (292-870)	703 (306-1074)	1236 (460-4716)

X- axes shows the number of CD34x10⁶/L blood when harvest was started

a) Total number of apheresis per patient

b) Total number of stem cells collected per patient. Mean value and range in parenthesis

Figure 3. shows the number of CD34+ cells at the first day of harvesting, total number of aphaeresis procedures and the total number of stem cells collected.

Table I. Total number of patients, number of CD34 analysis and number apheresis procedures performed for the first three years.

Year	Number of patients	Number of CD34+ blood analysis	Number of apheresis procedures
2004	16	80	42
2005	12	71	29
2006	20	83	42
Sum	48	234	113

Table II. Clinical characteristics and number of patients where cells were harvested and reinfused respectively

Diagnosis	Number of patients harvested	CD34+ cells collected	Number of patients reinfused	CD34+ cells reinfused
Lymphoma	21	9,2 *) (3,0-31,0)	14	4,1 (2,0-9,6)
Hodgkin's disease	6	7,9 (4,5-15,3)	4	3,9 (2,1-5,3)
Myeloma	15	8,6 (3,0-15,3)	15	3,2 (1,9-5,6)
Leukemia	3	22,6 (3,8-54,5)	0	
Total	45	9,8 (3,0-54,5)	33	3,7 (1,9-9,6)

*) CD34x10⁶/kg, mean and range in parenthesis

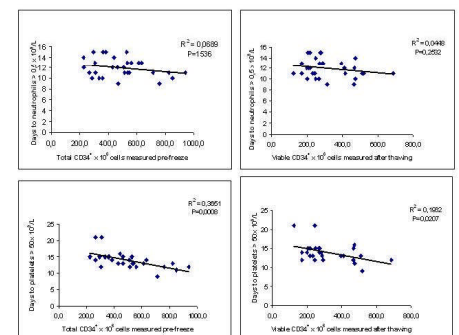


Figure 4. Correlation between infused CD34+ cells and engraftment kinetics (days until reconstitution) for 33 patients. The left and the right part of the figure shows the total number of CD34+ cells determined in fresh (pre-freeze) and thawed samples respectively.

Summary

■ During the first three years of the program 48 patients started treatment. CD34+ cells were successfully harvested from 45 patients (94%).

■ The mean number of apheresis procedures was 2,5 / patient. 33 patients have been re-infused with all or part of their cells.

■ The mean engraftment time is comparable to what has been seen in other Nordic countries.