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CASE SERIES

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The Importance of Positive Immunomagnetic Cell Separation Prior to Autologous Hematopoetic Stem Cell Transplantation for Advanced Stage Lymphomas

István Benedek[1,2], Judit-Beáta Köpeczi[2], Enikő Kakucs[2], Szende Jakab[1,2], István Benedek Jr[1,2], Erzsébet Lázár[1,2]

[1] University of Medicine and Pharmacy, Tîrgu Mureș, Romania

[2] Clinic of Hematology and Bone Marrow Transplantation Unit, Tîrgu Mureș, Romania

CORRESPONDENCE

István Benedek Jr
Str. Revoluției nr. 35
540042 Tîrgu Mureș, Romania
Tel: +40 265 218 739
E-mail: benedekistvan73@yahoo.com

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István Benedek • Str. Gheorghe Marinescu nr. 38, 540139 Tîrgu Mureș, Romania. Tel: +40 265 215 551

Judit-Beáta Köpeczi • Str. Revoluției nr. 35, 540042 Tîrgu Mureș, Romania. Tel: +40 265 218 739

Enikő Kakucs • Str. Revoluției nr. 35, 540042 Tîrgu Mureș, Romania. Tel: +40 265 218 739

Szende Jakab • Str. Gheorghe Marinescu nr. 38, 540139 Tîrgu Mureș, Romania. Tel: +40 265 215 551

Erzsébet Lázár • Str. Gheorghe Marinescu nr. 38, 540139 Tîrgu Mureș, Romania. Tel: +40 265 215 551

ABSTRACT

We present the method of immunomagnetic stem cell separation with the ISOLEX 300i device (Isolex® 300i Magnetic Cell Selection System, Nextell Therapeutics Inc. Irvine California 21618 USA) and the results obtained using this method in patients admitted to the Hematology and Bone Marrow Transplantation Clinic of Tîrgu Mureș, Romania. Cell selection has a great importance in separating stem cells from tumor cells, therefore contributing to the success of autologous stem cell transplantation.

Keywords: immunomagnetic cell separation, autologous stem cell transplantation

INTRODUCTION

The role of immunomagnetic cell separation in autologous stem cell transplantation is to eliminate tumoral cells (purging) or to concentrate the target cells (e.g. stem cells, dendritic cells) with the purpose of ulterior *ex vivo* cell manipulation (e.g. cell cultures).

Immunomagnetic cell separation can be:

- positive: it targets the CD34+ stem cells, it does not depend on the properties of the tumoral cells or their surface markers, and it results in the positive elimination of tumoral cells;
- positive-negative: it targets the tumoral cell and it is indicated in cases where the harvested product is highly contaminated with tumoral cells; the purity of the final product after this method is higher.[1]

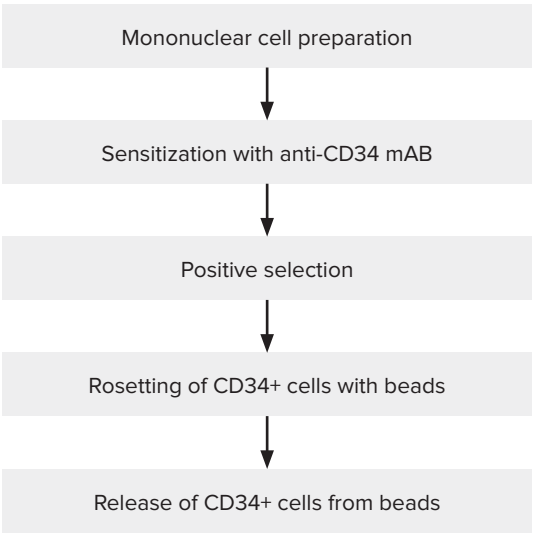

FIGURE 1. The phases of immunomagnetic cell selection

Immunomagnetic cell separation can be performed only in cases in which the product — obtained after the harvesting process by cytapheresis — contains a sufficient number of CD34+ stem cells, because with this method the cell loss can be around 10–40% per procedure.

By using different methods for CD34+ cell selection, it can be demonstrated that CD34-selected autograft cells are capable of long-term engraftment.

METHODS

The cell separation technique has been implemented at the Hematology Clinic and Bone Marrow Transplantation Unit since 2001, with the use of the ISOLEX 300i cell separator (Isolex® 300i Magnetic Cell Selection System, Nextell Therapeutics Inc. Irvine California 21618 USA).

In this article, we highlight the utility of this method by presenting several patients who had undergone this procedure.

The following reagents are necessary for positive immunomagnetic selection: mouse anti-human CD34 monoclonal antibody (9C5), sheep anti-mouse immunomagnetic beads (SAM), the releasing agent of stem cells being an oligopeptide that has the role to specifically release the CD34+ antigen. In positive-negative selection, besides these reagents, specific antitumoral reagents are also used (e.g. anti CD20-B cells).

The phases of immunomagnetic cell separation are the following (Figure 1):[2]

1. Installing the kit on the ISOLEX device: the reagents and the mononuclear cell product (containing the stem cells) obtained by cytapheresis; choosing the needed specific program of the device for positive or positive-negative selection.
2. Sensibilization (incubation) of CD34+ cells with mouse anti-human CD34+ monoclonal antibodies (9C5).
3. The sensitized CD34+ cells form the rosette with the immunomagnetic beads, which will be enhanced by the immunomagnetic part of the device. The rest of the cellular components are passively eliminated in a collection bag (waste-bag) resulting in the negative fraction, which contains tumoral cells. In the case of positive-negative selection, in this phase the sensitizing of target cells (tumoral cells) is carried out with specific monoclonal antibodies.
4. Under the action of the releasing agent, CD34+ cells are released from anti-CD34+ monoclonal antibodies and are collected in a separate bag. This will be the so-called positive fraction, which contains CD34+ cells with a very high grade of purity. This positive fraction will be cryopreserved at −190°C and used when needed for autologous stem cell transplantation. After the immunomagnetic cell separation, CD34+ cells do not contain

TABLE 1. The results obtained after positive immunomagnetic selection

Patient	Age (yrs)	Gender	Diagnosis	Treatment prior to autologous stem cell transplantation
I	25	F	B-cell non-Hodgkin lymphoma stage IV	chemotherapy + radiotherapy
II	52	M	Mantle cell B-cell non-Hodgkin limfoma stage IV	chemotherapy + surgical intervnetion to reduce the abdominal tumor mass + splenectomy
III	24	M	T-cell non-Hodgkin lymphoma stage IV	chemotherapy
IV	33	M	B-cell non-Hodgkin lymphoma stage IV	chemotherapy + monoclonal anti-CD20 antibody + radiotherapy
V	38	F	B-cell non-Hodgkin lymphoma stage IV	chemotherapy + radiotherapy
VI	44	M	B-cell chronic lymphocytic leukemia	chemotherapy + monoclonal anti-CD52 antibody
VII	41	F	B-cell non-Hodgkin lymphoma stage IV	chemotherapy + monoclonal anti-CD20 antibody + radiotherapy

F – female, M – male

TABLE 2. The results obtained after positive immunomagnetic selection

Patient	CD34+ × 10^6/kg bw before immuno-magnetic selection	CD34+ × 10^6/kg bw after immuno-magnetic selection	CD34+ (%) stem cell recovery after immuno-magnetic selection
I	19.85	16.48	83
II	10.33	8.27	80
III	8.64	5.62	65
IV	13.13	10.38	79
V	6.37	3.7	58
VI	5.6	2.8	50
VII	10.82	7.79	72

residual monoclonal antibodies and/or immunomagnetic beads on their surface. The surface antigens of CD34 cells remain intact.[3]

Immunomagnetic cell separation has several indications prior to autologus stem cell transplantatiom, such as: acute myeloid leukemias, malignant lymphomas, or neuroblastoma in children.[4]

The most rapid method to verify the content of CD34+ cells in the apheresis product is flow cytometry.[5]

CASE SERIES

We present several cases that have benefited from immunomagnetic cell separation, which has improved the outcome of autologus transplantation. We briefly present 7 cases in which we used immunomagnetic CD34+ selection prior to autologous transplantation to prevent the contamination of the apheresis products with malignant cells. All cases were stage IV lymphomas with different histological aspects. Due to the contamination of the apheresis product with malignant cells, a positive immunomagnetic selection was performed. The patients had been heavily pretreated and had received several lines of chemotherapy ± radiotherapy ± monoclonal antibody treatment prior to cell selection and autologous transplantation. Patient characteristics are presented in Table 1.

The purity of the final product after the positive immunomagnetic selection was very good in all cases, ranging from 96.12% to 98.38%, and the number of CD34+ cells/kg bw was sufficient in all the studied cases for successful autologous hematopoietic stem cell transplantation.

Table 2 presents the results of the positive immunomagnetic selection in the 7 studied patients.

Figure 2 shows the flow cytometric image of the graft contamination before and after the positive immunomagnetic stem cell separation. The apheresis product was contaminated before immunomagnetic selection with 40.2% malignant cells. After the immunomagnetic selection, the purity of the positive fraction containing the concentrated CD34+ stem cells was only 0.28% and the negative fraction contained a high grade of malignant cells, 39.09%.

In all cases a considerable reduction of tumoral contamination of the stem cell product was obtained, with values ranging between 3.3–4.1 logs, as shown in Table 3.

One of the risks of cell selection is the loss of CD34+ cells during the procedure, which can reach 40–50%. To prevent this loss, the method should be indicated in cases with a high number of CD34+ cells in the apheresis product, in order to have a sufficient number of CD34+ cells at

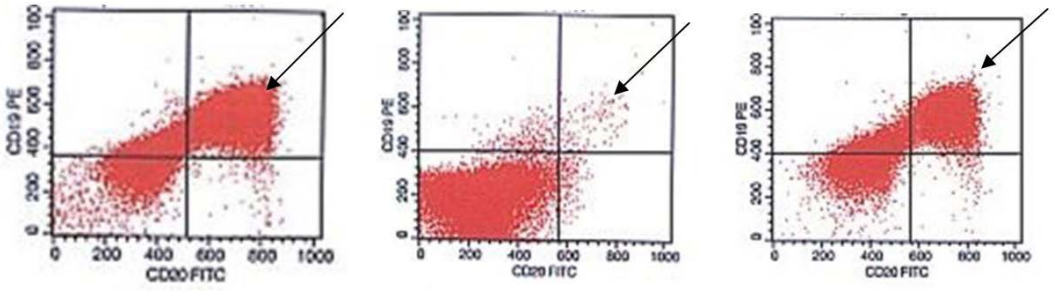

FIGURE 2. **A** – Before the immunomagnetic selection: the graft obtained by cytopheresis; **B, C** – After immunomagnetic selection: **B** – Positive fraction, **C** – Negative fraction

TABLE 3. The purity of the graft after positive immunomagnetic selection

Patient	The purity of the graft after CD34+ (%)	Depletion of the tumor cells (log)
I	98.37	4.0
II	98.11	4.1
III	96.12	3.4
IV	96.55	3.3
V	97.15	3.5
VI	97.31	3.8
VII	97.53	3.8

the end the procedure for a safe autologous transplantation. The minimal number of CD34+ cells required to perform autologous stem cell transplantation is 2.6×10^6/kg.

DISCUSSIONS

The role of cell manipulation and cellular therapy has significantly increased in the last decade.[6] Cell separation opened new horizons in immunotherapeutic strategies prior to or following stem cell transplantation. The separation of Natural Killer cells has a major role in enhancing the graft-versus-leukemia and -lymphoma effect.[7]

Separation and cell therapy with mesenchymal stromal cells facilitate engraftment and improve therapeutic results in case of steroid-resistant graft-versus-host disease (GVHD), even for very severe acute GVHD.[8]

Mesenchymal stem cells were also shown to induce proliferation and migration of normal and chronic wound fibroblasts and can enhance angiogenesis *in vitro*.[7]

The selection and administration of autologous T cells in certain malignant diseases (e.g. multiple myeloma) can be used for consolidation post-autologous transplantation, and the allotransplantation may not be necessarily performed, taking in consideration the very high morbidity and mortality of the procedure.[9]

Immunogenetic CD34+ cell selection has been introduced in the Hematology Clinic and Bone Marrow Transplantation Unit of Tîrgu Mureș with the aim to purify the graft to prevent relapse and to enhance a durable engraftment in stage IV non-Hodgkin lymphomas.[10,11]

The results obtained by this method are comparable with the results from very experienced transplant centers.[12] All the patients in this case series had a sufficient number of CD34+ cells after the positive selection, were successfully transplanted with quick engraftment, and presented tolerable complications, none of them being life-threatening.

CONCLUSIONS

The contamination of the stem cell final product with tumoral cells in considerably reduced after performing positive immunomagnetic cell separation.

The method contributes to the success of autologous hematopoetic stem cell transplantation, preventing relapse due to the important reduction of the number of malignant cells in the graft.

Autologous stem cell transplantation is easy to perform after immunomagnetic cell selection, due to the high purity of the graft (96–99%).

Immunomagnetic cell selection can be performed only in cases where cytapheresis leads to a sufficient number of CD34+ cells in order to perform a successful autologous stem cell transplantation, taking into consideration the possible cell loss during the procedure.

This method can be considered feasible and highly beneficial for the post-transplant evolution of patients with lymphoma in advanced stages.

CONFLICT OF INTEREST

Nothing to declare.

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