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Autologous and allogeneic transplantation with peripheral blood CD34+ cells: a pediatric experience

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ABSTRACT

Background and Objective. Peripheral blood stem cells (PBSC) have replaced bone marrow (BM) as the primary form for autologous hematopoietic stem cell transplantation. Furthermore, the use of allogeneic PBSC transplantation is now rapidly expanding and several centers have adopted this procedure. A new strategy in the use of PBSC is positive selection of CD34+ hematopoietic progenitor (CD34+) cells, and indeed large-scale devices for the clinical exploitation of CD34+ cell selection are now commercially available. In the autologous setting, the primary advantage of using CD34+ selected PBSC is reduced tumor cell contamination during PBSC preparation. On the other hand, in the allogeneic setting, CD34+ selection methods are used to reduce the incidence and severity of GvHD. Initial trials of CD34+ selected PBSC transplants have mainly been performed in adult cancer patients, and experience with CD34+ selected PBSC transplantation in pediatric populations is still limited. The purpose of this review is to clarify the status of CD34+ selected PBSC transplantation in the pediatric population.

Evidence and Information Sources. All authors of the present review work in the field of pediatric stem cell transplantation and in a stem cell processing laboratory, and have contributed to original papers published in peer-reviewed journals. The materials examined in the present review include articles and abstracts published in journals covered by the Science Citation Index® and Medline®. However, since there is still limited experience with CD34+ cell selection in pediatric populations, information on experience in adults will be discussed regarding the CD34+ cell-selection procedures and transplantation. Pediatric experience with transplants with CD34+ selected cells will be presented and discussed primarily based on our own experience. Specific problems related to PBSC mobilization and collection in children will also be discussed.

State of the Art. A review of the literature shows that with current CD34+ selection methods, purity of the CD34+ cell fraction can range from 30% to 90%, and two to three logs of T-cell depletion can be achieved. Tumor cell contamination has not yet been fully evaluated. The clonogenic activity of progenitor cells after CD34+ selection from PB remains high. Transplantation of autologous selected CD34+ cells from PBSC gives prompt and stable engraftment. The long-term therapeutic efficacy should be evaluated with regard to tumor recurrence. Allogeneic CD34+ selected cells successfully engraft immunomyeloablated recipients though a mega-cell dose effect between HLA-matched pairs. The results of allogeneic CD34+ selected cell transplantation from HLA-mismatched donors are, so far, not satisfactory because of the high rate of rejection, severe infectious complications and relapse of the disease. CD34+ selection may also be used as a target of gene therapy, as a source of dendritic cells for cancer immunotherapy and for the treatment of patients with autoimmune disease.

Key words: CD34+ hematopoietic progenitor cells, collection, purification, autologous and allogeneic transplantation, pediatric experience

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Hematopoietic stem cell transplantation (HSCT) is being increasingly used for the treatment of a variety of hematologic and oncologic disorders in childhood; aplastic anemia,[1] hemoglobinopathy,[2] congenital immunodeficiency,[3] selected acute and chronic leukemia[4-7] and cancer with a poor prognosis when treated with currently available multidisciplinary therapy, such as neuroblastoma and soft tissue sarcoma.[8-14] Autologous mobilized peripheral blood stem cell transplantation (PBSCT) results in rapid and durable trilineage hematopoietic recovery after myeloablative chemotherapy, and replaces bone marrow transplantation (BMT).[15,16] In BMT, the HSC that exist in the iliac bone can be collected by aspiration under general anesthesia. However, the hematopoietic activity in this area decreases with age, which very often makes the procedure inefficient. PBSC can be collected from the body's entire pool of HSC to provide more stem cells than can be obtained by localized BM aspiration from iliac bones; this leads to the faster recovery of hematopoiesis after PBSCT than after BMT, with fewer infectious complications,[17] and

makes *cell component therapy* far more effective with PBSC. Furthermore, the collection of PBSC does not require anesthesia or multiple marrow aspiration, and hence is far less invasive than bone marrow collection.[18]

Even in the field of allogeneic transplantation, the use of PBSC is rapidly expanding as a treatment for hematologic malignancies, and initial reports are encouraging.[19-21] The use of PBSC offers several advantages, for both donors and patients.[22] For donors, the harvest of PBSC does not require general anesthesia and does not cause local trauma, as mentioned earlier. For recipients, the use of PBSC results in faster engraftment, which may be associated with a better clinical outcome.[23] Although the risk of graft-versus-host disease (GvHD) is not increased despite the transfusion of a heavy load of lymphocytes, the impact on GvHD and graft-versus-leukemia (GVL) effect remains to be resolved.[24]

A newly developed strategy in the use of PBSC is the positive selection of $CD34^+$ hematopoietic progenitor ($CD34^+$) cells; large-scale devices for clinical $CD34^+$ cell selection are now commercially available.[25,26] The $CD34^+$ antigen is present on the earliest identifiable progenitor cells and committed myeloid precursors, whereas it is not expressed on mature hematopoietic cells or solid tumor cells.[27,28] Transplantation of $CD34^+$ selected cells offers several clinical advantages compared to conventional transplantation with buffy coated BM harvest or unmanipulated PB products in both autologous and allogeneic settings. In the autologous setting, the number of tumor cells contaminating the autografts can be reduced (up to four logs in solid tumors), without the use of pharmacologic agents such as 4-hydroxycyclophosphamide or immunotoxins.[29-31] In the allogeneic setting, T-cell depletion of up to four logs can be achieved.[32] After the positive selection of $CD34^+$ cells, the clonogenic activity of recovered progenitor cells remains high compared with that obtained after other methods of tumor cell purging, such as the use of maphosphamide or 4-hydroperoxycyclophosphamide,[29-31] or T-cell depletion via elutriation or negative selection using T-cell monoclonal antibodies. More importantly, PBSC may be easier to manipulate *in vitro* because of the number of progenitor cells available.[33]

Mobilization of PBSC

The mechanism of PBSC mobilization is not yet clear. Several cell adhesion molecules might be involved in PBSC mobilization.[34-37] PBSC mobilization in humans was initially noted during recovery after myelosuppressive chemotherapy.[38,39] Disease-specific chemotherapy has been used as well as a specific mobilization protocols.[40-42] In children, a rapid increase in the blood cell count in the recovery phase of chemotherapy predicts a higher cell yield by apheresis than in adult patients.[43] The main limitations of chemotherapy mobilization are neutropenia and the unpredictability of the timing of harvest. The ability of cytokines to mobilize blood cells, either alone or by enhancing chemotherapy mobilization, has been recognized. Granulocyte colony-stimulating factor (G-CSF) and granulocyte/macrophage colony-stimulating factor (GM-CSF) are now used in clinical PBSC collection.[44] The timing of harvest can be adequately predicted when mobilization with cytokine(s) alone is used. Many other cytokines with mobilization potential have been investigated, including stem cell factor (SCF),[45] macrophage inflammatory protein (MIP)-1α,[46] interleukin (IL)-1,[47] IL-3,[48] IL-6,[49] IL-8,[50] IL-11,[51] erythropoietin[52] and thrombopoietin.[53] Identification of the optimal cytokines and protocol for use in PBSC mobilization has become a major issue. A growing concern is that tumor cells may be recruited into the peripheral circulation by protocols used for HPC mobilization.[54]

In the allogeneic setting, G-CSF is exclusively used in normal healthy donors because it has fewer toxic effects than other available cytokines.[55] The optimal dose and schedule for the administration of G-CSF have not yet been established in children. In normal adult donors, a 5-day course of G-CSF at a dose of 10-12 μg/kg/day is widely used.[56] A lower dose might be able to induce a sufficient increase in circulating progenitor cells in children, considering their hematopoietic capacity. After daily G-CSF administration, the level of circulating $CD34^+$ cells usually peaks at around day 5, whereas leukocytosis is observed shortly after G-CSF administration.[57] G-CSF administration is well tolerated in children, and most children do not require analgesia to relieve bone pain.[58] In our preliminary study, side effects of G-CSF administration were rare in children compared to in adult donors (Table 1). Other common side effects include slight fever, general fatigue and asymptomatic elevation of serum alkaline phosphatase and transaminases.[59] Rare side effects such as splenic rupture,[60] iritis[61] and retinal hemorrhage[62] following G-CSF-administration have been reported. More importantly, with respect to donors who are children, we need to develop reliable and easy-to-use methods to reduce their anxieties about drug treatment, needle punctures, and the entire harvesting process. In pediatric allogeneic $CD34^+$ selected cell transplantation, most of the donors are adults because parents are often chosen as HLA two or three loci-mismatched donors when this procedure is limited to transplantation between HLA-mismatched related pairs.

Collection and cryopreservation of PBSC

Experience with PBSC collection from a pediatric population is still limited, especially from normal donors. The main distinctions of a pediatric population are the special requirements for vascular access[63-65] and leukapheresis, high progenitor yields, and the risk of stem cell exhaustion.[66] The yield of progenitor cells might depend on the speed at which blood is withdrawn.

Table 1. Occurrence of side effects related to G-CSF treatment in normal donors.

< 10 y.o. (no. 9)	No complaints	9/9
> 10 y.o. (no. 5)	Mild headache	3/5
	Lumbago	3/5
	General fatigue	2/5
Adult donor (no. 22)	Lumbago/headache	22/22
	Nausea	2/22
	Skin induration	1/22

Many centers use a tunneled, double-lumen Broviac catheter, which is inserted via subclavian veins or saphenous veins under general anesthesia.[63] A pediatric MedComp hemodialysis catheter or a temporary hemodialysis catheter is also used, and is inserted under conscious sedation.[67] We have used a temporary radial artery catheter which was inserted without any sedation or anesthesia.[68] In normal PBSC donors, it is common practice not to place a central line, but rather to use a peripheral line to avoid the risks involved with catheter placement.

Care should be taken regarding acid citrate dextrose (ACD)-A toxicity and hypovolemia when PBSC are harvested from children. Calcium replacement is preferred when ACD-A is used in large-volume leukapheresis to maintain acceptable levels of ionized calcium. The devices used for PBSC collection are Fenwall CS3000 Plus, AS100 and COBE spectra. Small volume collection chambers (SVCC) and small volume separating chambers (SVSC) are used to reduce the extracorporeal volume to 140 mL. We tested the efficacy of a new procedure involving SVSC plus SVCC in eliminating the abrupt change in blood volume during apheresis for small children by monitoring the intra-apheresis dynamics of hematocrit values, and found that the application of this procedure prevented a rapid change in the hematocrit level at the initiation of apheresis without reducing the collection efficiency.[69] Furthermore, the use of SVSC plus SVCC can reduce the volume of blood needed to preprime the machine. Priming with autologous blood or leukocyte-depleted red blood cells is used to reduce the risk of hypovolemia when collection is started when PBSC are collected from donors weighing less than 20 kg. Autologous blood is preferred for normal pediatric donors to avoid side effects caused by allogeneic blood transfusion. In our PBSC transplant units, we collect autologous blood once a week, beginning three or four weeks before apheresis. When blood is withdrawn the second or third time, previously saved blood is infused back into the donor and 1.5 or 2 times the volume of saved blood is withdrawn to prevent hypovolemia, and more fresh blood is saved. A total of 150 mL is saved one week before apheresis and used for priming in the first apheresis. At the end of the first apheresis, any blood remaining in the machine is collected using the collection mode, and used for priming in the second PBSC collection.

In the autologous setting, collected PBSCs must be frozen until infusion after preparative high-dose chemotherapy. PBSC can be frozen by a simplified procedure without a programmed freezer using 6% hydroxyethyl starch (HES) and 5% DMSO, without losing their clonogenic viability or engraftment potential.[70] Frozen PBSC are stored in the liquid phase of liquid nitrogen or in an electric freezer at –135°C. In the allogeneic setting, PBSC are not always frozen. Fresh PBSC can be infused into patients who have completed preparative chemoradiotherapy. However, it is rather difficult to assess the quality of PBSC, and the effects of this approach on the incidence and severity of GVHD remain to be determined.

Enrichment of CD34+ cells

The CD34 antigen is a 115-kDa surface glycophosphoprotein that is expressed on 1-3% of normal bone marrow cells, including both committed and probably long-term reconstituting progenitor cells,[27] whereas it is not expressed on mature hematopoietic cells or solid tumor cells.[27,28] The development of monoclonal antibodies that identify different epitopes of the CD34 antigen has led to several immunologic techniques for the positive selection of cells labeled with an anti-CD34 monoclonal antibody.

Negative selection can be used to deplete high levels of tumor cells. However, there can be significant losses of progenitor cells, and purging by chemical agents can damage progenitor cells.[29-31] Enrichment of CD34+ cells provides an attractive alternative, in that several systems are available commercially and these are usually automated and relatively rapid, making daily use more feasible[71-74] even in children.[75,76] The different devices available have not yet been directly compared for PBSC, although such a comparison has been made for BM cells.[77,78] In our cell-processing laboratory, the leukapheresis product obtained on the first day was stored at 4°C overnight and pooled with the product obtained on the second day.[79] The two aphereses products were then processed on an Isolex 300 (Baxter Healthcare Corp., Irvine, CA, USA) at the same time. The removal of platelets before application of the anti-CD34 antibody partly prevented the binding of stem cells by anti-CD34 antibody. It took about three hours to isolate CD34+ cells. The average purity of the CD34+ cell fraction was 79%, with an average recovery rate of 21%. Cells were mixed slowly with an equal volume of a freezing solution containing 8% human albumin, 12% HES, and 10% dimethylsulfoxide (DMSO) to give final concentrations of 6% HES and 5% DMSO. Both CD34-positive and -negative cells were transferred to 5-mL polypropylene tubes and then placed

directly in an electric freezer that maintained a temperature of –135°C (Sanyo Electric Co., Tokyo, Japan). The cells were stored in the same freezer until use. With the currently available techniques, the purity of the CD34+ selected cells varies between 30% and 90%, which equates to detectable levels of tumor cells. Clonogenic activity after CD34+ cell selection remains high. Significant cell loss occurs, which increases the number of aphereses needed to collect sufficient CD34+ cells. However, when allowance is made for the number of CD34+ cells infused, the use of purified CD34+ cells is not associated with any delay in engraftment.[25,80,81] G-CSF-mobilized PB leukapheresis products undergoing the selection of CD34+ cells give a greater yield and enrichment of progenitor cells than BM harvest collected from HLA-identical normal healthy donors for allogeneic transplantation.[82] However, considering the cost and time needed for CD34+ cell isolation, some researchers claim that the benefits of isolation remain unclear.

Other benefits of CD34+ cell purification include avoiding the toxicity due to DMSO and cell lysis products.[83] Furthermore, the reduced volume of the infusion makes it feasible to infuse the grafts directly into BM to avoid trapping stem cells in the lungs.[84-86] We hypothesized that direct puncture of the marrow cavity to implant a graft, rather than systemic intravenous administration, may guarantee more stable engraftment in clinical transplantation, but only when cells are purified to reduce the total volume of the graft.[87]

The number of CD3+ cells in the CD34- fraction should be sufficient to allow for multiple graded incremental T cell aliquots for donor lymphocyte infusion (DLI).[88] An unadsorbed fraction containing 85% functional T cells may be stored in graded aliquots to support post-transplant immunotherapy, when necessary. This strategy may be the preferred therapeutic option in patients considered to be at high risk of relapse.

However, the use of CD34+ cells as a marker for HSC has recently been questioned.[89] More primitive progenitor cells might exist in the CD34- fraction.[90]

Autologous transplantation with CD34+ selected cells

The clinical relevance of tumor cell contamination within autologous HPC grafts as a source of relapse remains controversial,[91-93] and as yet no adequately sized trials have addressed this point. CD34 selection might not eliminate contaminating tumor cells.[94,95] The assessment has been hampered by the lack of sensitive clonogenic assays to detect residual tumor cells.[96,97] With the advent of molecular assays for the detection of residual tumor cells, this assessment may become feasible and readily available. Using either sensitive immunofluorescence techniques or the polymerase chain reaction (PCR), neuroblastoma cells have been detected in virtually all PBSC products,[98] and these quantities are probably sufficient to contribute to relapse after transplantation.[99] For some patients, CD34+ selection alone is not sufficient to render PBSC products tumor cell-negative, and for these patients, additional tumor cell depletion may be necessary.

Although potential disadvantages of purified CD34+ cells are that the reconstitution of hematologic function may be delayed due to the lack of facilitating cells[100] and the susceptibility to damage inflicted by cryopreservation and thawing, the use of purified CD34+ cells is not associated with any delay in engraftment. Initial trials of autografts with CD34+ selected PBSCs have mainly been performed in adult patients.[80,81,101] The reasons for the limited use of this technique in children have already been addressed. In our study on pediatric patients, we compared engraftment days between different modes of transplantation with purified or unmanipulated blood cells (Table 2). Immunologic recovery after autologous CD34+ selected cell transplantation has not yet been reported. In our series of studies, we compared lymphocyte phenotypes after autologous CD34+ selected cell transplantation with those after autologous unmanipulated cell transplantation, and found no differences between the two types of transplantation (unpublished data).

A longer follow-up will be required to assess the role and impact of CD34+ selection on the outcome of high-dose therapy. There are on-going randomized trials evaluating enriched CD34+ cells autotransplants in adult patients with cancer such as breast cancer and multiple myeloma.[101-103]

A very intriguing approach is consecutive high-dose therapies for childhood cancer, in which each course is followed by transplantation with G-CSF-mobilized PBSC that have been been separated into CD34-negative and -positive fractions.[104] The CD34-negative fraction is used for the first transplantation and the CD34-positive fraction is used for the second transplantation. The objectives of this approach are to

Table 2. Comparison of engraftment days between different modes of transplantation with purified or unmanipulated blood cells.

	Auto PBSCT (no.= 72)	*Auto CD34+ (no.= 20)*	*Allo-PBSCT (no.= 9)*	*Allo CD34+ (no.= 8)*
AGC >500 (x10^6/L)				
Median	12 (16-25)	11 (9-18)	10 (8-19)	14 (9-20)
Mean±SD	13±4	12±2	10±2	13±3
PLT >50 (x10^9/L)				
Median	16 (10-195)	26 (13-55)	16 (12-39)	20 (12-23)
Mean±SD	30±36	28±12	20±10	21±9

AGC: absolute granulocyte count; PLT: platelets.

enhance tumorcidal activity with double high-dose chemotherapy and to use progenitor cells in the CD34-negative fraction effectively. Interestingly, the CD34-negative fraction was able to support myeloablative chemotherapy, and there was no difference in engraftment speed between CD34-negative and -positive transplants.

Transplantation of allogeneic selected CD34+ cells

G-CSF-mobilized PBSC contain approximately one log more T-lymphocytes than BM.[105] Since T-lymphocytes are responsible for GvHD, a potential major disadvantage of allogeneic PBSCT is the possibility of an increased rate of GvHD compared with that occurring after allogeneic BMT. However, acute GvHD following unmanipulated allogeneic PBSCT between HLA-identical pairs does not seem to be increased, according to several recent studies.[19-21] This suggests that above a particular T-cell threshold, the specificity of T-cells for genetic disparity between the donor and recipient, rather than the absolute number of T-cells, determines the risk of GvHD. Thus, selection for CD34+ cells may not be required in allogeneic PBSCT between HLA-identical pairs, although chronic GvHD might occur at a high incidence following unmanipulated allogeneic PBSCT.[106-108] However, GvHD might contribute to a low relapse rate. This issue should be resolved in future studies.

One major limitation of allogeneic PBSCT is the lack of suitable donors. The search for an unrelated matched donor is time-consuming, and rapid disease progression in some patients makes this approach impractical.[109,110] The use of an HLA-mismatched related donor avoids the lengthy search procedure and provides donors for 90% of patients who may potentially benefit from allogeneic transplantation. To reduce the potential risk of severe GvHD, especially in transplantation between HLA-mismatched related pairs, several investigators have attempted to remove T-lymphocytes from allogeneic grafts using the positive selection of CD34+ cells.[111] This removes a median of 2 to 2.5 log of T-cells while retaining 40% to 70% of the CD34+ progenitor cells. An advantage of CD34+ cell selection over antibody purging or elutriation of T-cells is that a compound allograft is produced, consisting of stem cell-enriched and unadsorbed fractions, the latter containing T-cells which may be used for post-transplant immunotherapy.

In allogeneic CD34+ selected cell transplantation between HLA-matched pairs, rapid early and durable engraftment has been achieved.[112-115] Chimerism studies in allogeneic CD34+ selected PBSCT have shown that complete chimerism is achieved in recipients of a large number of CD34+ cells.[116,117] Furthermore, to achieve complete chimerism, intense myeloablative treatment including total body irradiation (TBI) might be necessary, especially in transplantation between HLA-mismatched pairs. So far, this procedure has been shown to be likely to prevent GvHD.[118] However, there are controversial reports that question whether CD34+ cell selection combined with post-transplant cyclosporin A (CsA) with or without methotrexate is sufficient prophylaxis against acute GvHD.[111]

Profound post-transplant immunodeficiency after allogeneic CD34+ selected cell transplantation may lead to a high risk of lethal infectious complications[119-121] and post-transplant lymphoproliferative disorders.[122] The immunodeficiency seen after T-cell-depleted transplantation including CD34+ selected cells is caused by qualitative defects in cellular and humoral immunity that can not be simply explained by deficits in the numbers of circulating lymphocytes. One possible explanation may be the decreased diversity of the T-cell repertoire after transplantation.[123] However, since additional B-cell depletion might be beneficial for reducing post-transplant lymphoproliferative disorders,[122] positive selection of CD34+ cells may be a better approach than other methods of T-cell depletion. The increased incidence of leukemia relapse after T-cell depletion will obviously not be overcome with the use of CD34+-selected PBSC alone. However, the large quantities of lymphocytes that can be segregated from PBSC grafts might be used to add GVL activity with delayed T-cell addbacks. Optimizing the dose and the timing of T-cell addback, and the method of GVHD prophylaxis should be evaluated.[124]

In our phase I feasibility study in patients who lacked an HLA-matched donor, 13 children were enrolled and PBSC were collected from healthy mismatched family donors who varied in one (2), two (9) or 3 loci (2) from the respective recipients.[32] Subsequent bulk depletion of T-cells from PBSC was accomplished with an ISOLEX 300 system. The median number of cells subjected to enrichment was 3.8×10^{10} (range 1.2-10.4), and 2.63×10^{8} (range 0.14-3.65) CD34+ cells were recovered with a median purity of 80% (range 19-98). The median yield of CD34+ cells and CFU-GM was 37% and 28%, respectively. Consequently, an average of 7.0×10^{6}/kg (range 2.2-14) CD34+ cells and 0.97×10^{5}/kg (range 0.05-2.09) CD3+ cells were infused. One patient died of veno-occlusive disease of the liver (VOD) on day 17 and another patient in refractory leukemic relapse rejected the graft after transient neutrophil recovery. Nine of 11 patients demonstrated signs of engraftment. However, subsequent rejection was seen in 3 patients, two of whom had autologous recovery. Consequently, eight patients were evaluated in the early phase of marrow recovery. The median number of days to achieve an absolute granulocyte count (AGC) of 0.5×10^{9}/L was 14 (range 9-20) and that to achieve a platelet count of 50×10^{9}/L was 20 (range 12-23). Donor chimerism persisted in 5 patients until death or current survival. All of the surviving patients with functioning-donor-type hematopoiesis were giv-

en TBI. *De novo* acute GvHD (grades II and IV) was observed in 2 of the 8 evaluated patients. Scheduled donor lymphocyte infusion (DLI), using the CD34-negative fraction, was given to four patients, free of *de novo* acute GvHD, beginning 28 to 43 days following transplant. Three of these patients developed acute GvHD (grades I, II and IV).

Thus, this approach for $CD34^+$ cell purification should be carefully evaluated in the setting of HLA-mismatched PBSC transplantation and any benefits should be weighed against the potential increased risk of disease relapse, and perhaps delayed immunologic reconstitution, as well as the cost of the procedure.[125] A larger group of patients will have to be observed to answer these questions.

Promising future clinical applications

PB $CD34^+$ cells are attractive targets for gene therapy, although many problems must be resolved before this can become a clinical reality. Mobilized PB $CD34^+$ provide more progenitor cells than BM harvest. A high transduction efficacy has been demonstrated using $CD34^+$ selected PB cells.[126] However, continued expression post-transplantation remains low.

There has been interest in the *in vitro* production of dendritic cells (DC) from PB $CD34^+$ cells for use in tumor immunization programs.[127-130] $CD34^+$ progenitors are isolated from the blood of healthy donors and patients mobilized with G-CSF, and cultured in the presence of GM-CSF and IL-4 with or without tumor necrosis factor (TNF)-α or SCF.[132] Potential immunotherapy would involve *ex vivo* exposure of DCs from cancer patients to tumor antigen, and reinfusion of these pulsed DC into the patients.[133,134]

In various animal models, HSCT can be used to treat autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, basing this treatment on the hypothesis that autoimmune diseases originate from defects in hematopoietic stem cells.[135] Using $CD34^+$ cell purification, we can expect this approach to become a valuable strategy for the treatment of patients with autoimmune disease.[136-140]

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