

Graft engineering for autologous stem cell transplantation

Review Article

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Abbreviations: absolute lymphocyte count, (ALC); absolute lymphocyte count at day 15, (ALC-15); acute myelogenous leukemia, (AML); allogeneic stem cell transplantation, (Allo-SCT); autograft absolute lymphocyte count, (A-ALC); autologous stem cell transplantation, (ASCT); graft versus host disease, (GVHD); graft versus tumor, (GVT); granulocyte colony-stimulating factor, (G-CSF); granulocyte-macrophage colony-stimulating factor, (GM-CSF); minimal residual disease, (MRD); multiple myeloma, (MM); natural killer, (NK); non-Hodgkin's lymphoma, (NHL); overall survival, (OS); peripheral blood ALC at the time of collection, (PC-ALC); peripheral blood stem cell collection, (PBSC); progression-free survival, (PFS); stem cell factor, (SCF); stromal cell-derived factor-1, (SDF-1); vascular adhesion molecule-1, (VCAM-1)

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Summary

To date, the main therapeutic goal of the stem cell autograft has been the collection of sufficient stem cells in order to achieve hematologic engraftment following high-dose chemotherapy in the setting of autologous stem cell transplantation (ASCT). Efforts to improve the clinical outcomes of ASCT have recently focused on the impact of autograft contaminating tumor cells and methods related to their removal (purging). In the last five years, emerging evidence suggesting the impact of autograft lymphocyte content on post-ASCT immune reconstitution with dramatic improvement on clinical outcomes suggests a critical role for autograft immune cell content. Herein we review the clinical evidence suggesting that autograft engineering directed at immune cell enrichment may directly impact clinical outcomes in patients undergoing ASCT for malignant disorders.

I. Introduction

We currently accept that the clinical efficacy of autologous stem cell transplantation (ASCT) in treating malignancies relies entirely on the ability of the high-dose chemotherapy to eradicate tumor cells. To overcome the myelosuppressive effect of high-dose chemotherapy in ASCT, stem cells are harvested pre-treatment and re-infused into the patient to repopulate the bone marrow, leading to hematologic engraftment and reconstitution (rescue). The preferable modality of stem cell collection is via the peripheral blood (PBSC) over bone marrow harvest because of faster platelet and neutrophil engraftment (Shpall et al, 1999). The stem cell autograft in ASCT is mainly viewed as a procedure to collect enough CD34+ stem cells to achieve hematologic engraftment.

However, despite high-dose chemotherapy, ASCT is still not curative for a number of patients with malignant

diseases. One hypothesis for the lack of better clinical outcomes with ASCT has been the identification of contaminated tumor cells harvested during stem cell collections. Another possible mechanism for the high relapse rate observed in ASCT is the presumptive lack of graft-versus-tumor (GVT) present in allogeneic stem cell transplantation (Allo-SCT), where it is associated with a significant therapeutic impact on minimal residual disease (MRD) (Horowitz, et al, 1990). Recent reports have shown that early post-transplant lymphocyte recovery has a direct impact on survival in patients undergoing allo-SCT (Pavletic et al, 1998; Powles et al, 1998)). Likewise, it appears that early post-transplant lymphocyte recovery is equally (if not more) effective in improving clinical outcomes of patients post-ASCT, suggesting the existence of an autologous graft versus tumor effect (Porrata et al, 2004a). It appears that the source of early lymphocyte

engraftment post-ASCT is the lymphocyte, not CD34 cell content of the autograft (Porrata et al, 2004b). Thus, manipulations (engineering) of the autograft lymphocyte content may have a direct impact on clinical outcomes in ASCT. In this review we present clinical evidence of the role of the stem cell autograft as an immunologic adoptive transfer maneuver to achieve immunologic engraftment and improved survival post-ASCT.

II. Stem cell collection

Until 1986 the main modality of stem cell collections for ASCT was bone marrow harvest (Kessinger et al, 2003). An important discovery in the mid 1980's was the demonstration that stem cells could be harvested from the peripheral blood after mobilization with chemotherapy or growth factors such as granulocyte-colony stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kessinger et al, 1986; Korbling et al, 1986; Reiffers et al, 1986). Since then, peripheral blood stem cell collection (PBSC) has become standard of care for ASCT. This is not only because of greater convenience for the patient, but also because of increased CD34+ stem cell content of the autograft leading to more rapid hematologic engraftment (Sheridan et al, 1992; Bensinger et al, 1995; Schmitz et al, 1996).

A. Mechanisms of stem cell mobilization

The process of mobilizing stem cells from the bone marrow into the peripheral blood for its use in ASCT is not completely understood. Several studies suggest that stem cell mobilization involves adhesion molecules, cytokines, chemokines, and proteases.

Circulating stem cells express a variety of adhesion molecules including integrins, selectins, and selectin ligands (Lund-Johansen et al, 1993; Saeland et al, 1992; Zannettino et al, 1995). The down-regulated expression of CD49d (VLA-4), CD49e (VLA-5), and L-selection in the mobilized peripheral blood stem cells compared to steady-state bone marrow CD34+ stem cells indicates that down-modulation of adhesion molecules may be a factor in the release of stem cells from the bone marrow (Dercksen et al, 1995; Kroger et al, 1998; Prosper et al, 1998; Williams DA et al, 1991). In vivo evidence of the role of adhesion molecules in the release of stem cells into the peripheral blood came from the treatment of mice with antibodies to VLA-4 and vascular adhesion molecule-1 (VCAM-1) resulting in mobilization of CD34+ stem cells into the peripheral blood (Simmons et al, 1992; Papayannopoulou et al, 1995, 1998; Craddock et al, 1997).

Cytokines can also act as an adhesion molecule if present as a membrane-bound form. The receptor for stem cell factor (SCF, c-kit ligand) has been found to be expressed on the cell surface of bone marrow stromal and endothelial cells (Flanagan et al, 1991; Broudy et al, 1994). Low level expression of c-kit on peripheral blood CD34+ stem cells may suggest that the loss of this receptor facilitates the migration of stem cells from the bone marrow (Mohle et al, 1993).

The receptor for the chemokine stromal cell-derived factor-1 (SDF-1) is CXCR4. CD34+ stem cells express

CXCR4 enabling them to migrate across a gradient of SDF-1 concentrations (Mohle et al, 1998; Petit et al, 2002). The reduced ability of circulating stem cells to migrate in response to SDF-1 (Aiuti et al 1997) and reduced hematopoiesis in SDF-1 knockout mice suggest that SDF-1 affects transendothelial migration and may play a role in stem cell mobilization (Nagasawa et al 1996). In non-Hodgkin's lymphoma (NHL) patients, the expression of CXCR4 on CD34+ cells from the peripheral blood apheresis collections was found to be decreased compared to steady-state bone marrow stem cells (Gazitt et al, 2001). In this same study, patients that were 'good mobilizers' had decreased levels of plasma SDF-1 in the peripheral blood apheresis collections compared with 'poor mobilizers' patients. Thus, desensitization of the SDF-1 CXCR4 signaling pathway is required for successful CD34+ cell release from the bone marrow to the peripheral blood and can predict for good mobilization (Gazitt, 2004).

Neutrophil elastase and cathepsin G are the primary proteases associated with the release of stem cells to the circulation in mice, monkeys, and humans (Levesque et al, 2002a; Valenzuela-Fernandez et al, 2002). These proteases have been found to degrade CXCR4 and SDF-1 (Levesque et al, 2002b and 2003). Hence, proteases may contribute directly to desensitization of the CXCR4/SDF-1 signaling pathway and to the release of stem cells from the bone marrow (Gazitt, 2004).

B. Stem cell mobilization agents

Stem cell mobilization agents alter the steady-state balance in the bone marrow microenvironment by disrupting the interactions between chemokines, proteases, and adhesion molecules. Cytokines alone or in combination with chemotherapy have been shown to change the bone microenvironment promoting stem cell mobilization into the peripheral blood. Recently, a CXCR4-blocking molecule (AMD3100) has been developed to enhance stem cell mobilization and collection.

1. Cytokines

Human granulocyte colony-stimulating factor (G-CSF) alone or in combination with other cytokines/chemokines/chemotherapy is considered the cytokine of choice for stem cell mobilization. Candidates for the use of G-CSF alone for stem cell mobilization are normal donors, where chemotherapy is contraindicated, and autologous donors with malignancies in remission who might benefit by avoiding the risks of myelosuppressive chemotherapy (Kessinger et al, 2003). Using 16 µg/kg/day G-CSF in patients with hematologic malignancies or solid tumors, a 10-fold increment of peripheral blood CD34+ stem cells concentration over baseline has been observed, reaching peak values at about day 5 of G-CSF administration (Bensinger et al 1993). Higher dose of G-CSF (16 µg/kg) compared to lower dose (8 µg/kg) has been found to be more effective in mobilizing stem cells in patients with different malignancies (Demirer T et al, 2002). In comparison to G-

CSF, GM-CSF appears to be less effective in mobilizing stem cell (Peters et al, 1993; Lane et al, 1995). Other cytokines has been used in combination with G-CSF or alone as autologous stem cell mobilizers including SCF (Stiff, 1999), erythropoietin (Kessinger et al, 1995), interleukin-3 (Ohi et al, 1995), FLT3 ligand (Lebsack et al, 1999), and Interleukin 6 (Pettengell et al, 1995). Nevertheless, G-CSF has demonstrated superior results in mobilizing stem cells into peripheral blood and remains the mainstay of therapy.

2. Chemotherapy

Chemotherapy has also been used in combination with G-CSF or GM-CSF to mobilize stem cells. Initial reports demonstrated up to 1,000 fold improvements in CD34+ stem cell content in peripheral blood of patients mobilized with chemotherapy and growth factors as compared to growth factors alone (Demuyne et al, 1995; To et al, 1997). Besides the benefit of greater CD34+ stem cell concentration per liter of blood processed, reduced number of apheresis sessions and decreased numbers of contaminating tumor cells have been the main arguments for the use of chemotherapy based mobilization strategies (Negrin et al, 1995). However, these benefits need to be balanced with side effects of chemotherapy including neutropenia, thrombocytopenia, as well as non-hematologic toxicities (e.g. infections). Likewise, not all chemotherapy assisted stem cell mobilization is superior to G-CSF mobilization alone. A recent randomized study in patients with lymphoma did not find any difference in the CD34 stem cell content of the autograft in patients mobilized with G-CSF alone versus cyclophosphamide plus G-CSF (Milone et al, 2003). Thus, in most cases the use of chemotherapy may not be beneficial.

3. Novel agents

AMD3100 is a reversible inhibitor of the binding of stromal derived factor (SDF-1) to its cognate receptor CXCR4. It is the first agent to be studied for stem cell mobilization based on our understanding of its mechanism of action relative to stem cell-stroma interaction. While AMD3100 mobilizes stem cells on its own (Devine et al, 2004), it significantly enhances the mobilization capability of G-CSF (Pelus et al, 2002). Clinical trials in humans are in progress in different diseases, including NHL and multiple myeloma (MM).

C. Stem cell dose

The main goal of harvesting enough CD34+ stem cells in ASCT is to overcome the myelosuppressive effect of high-dose chemotherapy, resulting in hematologic engraftment (recovery of white cells, red cells, and platelets).

Several factors has been reported to predict hematological engraftment after ASCT including patient age, underlying disease, mobilization regimen, pre-apheresis patient blood counts, total autograft CD34+ stem cell content, and CD34+ cell subset dose (CD34+33+ and CD34+33- cell dose) (Pecora, 1999). Of these factors, the strongest predictor of hematologic engraftment is the total dose of infused CD34+ stem cells (Pecora, 1999). In a

study of 375 consecutive autologous stem cell transplant patients, the CD34+ stem cell dose was a highly significant factor ($p < 0.001$) predicting hematologic engraftment rates (Pecora et al, 1995). Patients receiving < 1 to 2×10^6 CD34+ cells/kg are at risk of engraftment failure (Kiss et al, 1997; Shpall, 1999). Faster neutrophil and platelet engraftment has been reported as the CD34+stem cell dose was increased from 2.5 to 5×10^6 CD34+cells/kg (Bensinger et al, 1995; Weaver et al, 1995). Further increasing the dose of autograft CD34+ stem cells has not translated into more rapid hematologic engraftment (Siena et al, 2000). Hence, the currently recommended target CD34+ cell dose of 5×10^6 /kg. The data suggest that a minimum of 7 days to achieve a neutrophil count of 500 cells/ μ l and 12 days for 50,000 platelets/ μ l will be observed after high-dose chemotherapy regardless of further CD34 dose escalation (Pecora, 1999).

Of note, similar to allo-SCT where the CD34 cell dose appears to directly affect survival (Perez-Simon et al, 2003; Ringden et al, 2003; Lee et al, 2005), in ASCT there has been one study showing that infusion of more CD34+ stem cells (6.1×10^6 CD34+ cells/kg) resulted in superior progression-free survival in NHL patients (Blystad et al, 2004). The underlying mechanism of why higher CD34+stem cells affect survival in the ASCT is not understood and further studies are warranted.

III. Autograft purging of contaminating tumor cells

Autologous stem cell transplantation (ASCT) improves survival of patients suffering from hematological malignancies (Philip et al, 1995; Attal et al, 1996). However, recurrence of disease after ASCT remains the major cause of treatment failure (Shimoni et al, 2002). Re-infusion of small numbers of viable clonogenic tumor cells contaminating the stem cell graft has been attributed as one possible factor responsible for the observed high rates of tumor recurrence (Jansen et al, 1984). To minimize the infusion of contaminating tumor cells in the stem cell autograft, different purging techniques has been developed to reduce the tumor content in the autograft (purging).

A. Gene-marking studies

To demonstrate that post-ASCT tumor relapses originate from autograft contaminating tumor cells gene marker studies have been performed in which self-replicating cells of the autograft are genetically labeled and the presence of this label is subsequently determined in the relapsed tumor (Shimoni et al, 2002). The most commonly used genetic marker has been the neomycin resistance phosphotransferase gene (*neo*). The first clinical study suggesting that tumor cell-contaminated autograft contributed to relapse reported four children relapsing after autologous stem cell transplantation for acute myelogenous leukemia (AML) (Brenner et al, 1993). The post-transplant relapsed leukemic blasts of these four patients formed neomycin-resistant colonies and the *neo* gene sequences were detected. Similar findings were reported in children transplanted for neuroblastoma (Rill et al, 1994) and patients with chronic myelogenous leukemia

(Deisseroth et al, 1994). However, other studies have reported non-detectable gene-marked tumor cells after relapse from ASCT (Dunbar et al, 1995; Cornetta et al, 1996; Bachier et al, 1999). Thus, marked tumor cells are not necessarily the sole cause of relapse, as remaining minimal residual disease after high-dose chemotherapy in the host may also contribute.

B. Purging techniques

Based on the association of re-infused tumor cells giving rise to at least part of post-ASCT tumor relapses it has been proposed that autografts be purged of contaminating tumor cells with the goal of at least 6-log reduction of tumor cells while sparing sufficient hematopoietic progenitor cells to allow engraftment (Batista et al, 2003). The current purging techniques are able to reduce between 2 to 6 logs of tumor contamination in the autograft (see below). Purging techniques include immunological, pharmacological, photosensitizing agents, physical and membrane-active agents, molecular biological and gene transduction approaches, and immune effector cells.

1. Immunological purging techniques

The immunological approach exploits the ability of murine monoclonal antibodies (Mabs) to bind antigens expressed on tumor cells but not on normal marrow stem cells (Jansen et al, 1984). The removal of antidody-coated cells can be achieved by direct killing, using complement-mediated cytotoxicity or specifically constructed immunotoxins (Mab coupled to drugs or toxins like ricin), or, by coupling Mabs to magnetic microspheres, antibody-coated cells can be physically removed from marrow suspensions by placement in a strong magnetic field (Jansen et al, 1984). If tumor cells are removed physically, the procedure is referred to as “negative selection”. Positive selection is the physical removal of CD34+ stem cells from the autograft. However, increased infection rates have been reported in CD34 positive selection purging techniques thought to be due to delayed immune recovery compared to un-manipulated autografts (Miyamoto et al, 1998; Friedman et al, 2000). These reports imply that the infused lymphocytes from the stem cell autograft might impact on post-transplant infection control. In addition the possible benefit derived from CD34 positive selection purging technique might be overcome by higher infectious morbidity and mortality (Rutella et al, 2001). In an attempt to compensate for the shortcomings of pure positive selections, combinations of negative and positive CD34+ cell selection have been undertaken (double purging) (Hoppe et al, 1999). Also, two-step negative depletion procedures have been described by initially debulking tumor cells using immune rosetting, followed by physically removing tumor cells through a depletion column (Rambaldi et al, 1998).

With the advent of rituximab (a human/mouse monoclonal antibody against the antigen CD20 expressed in 90% of NHL patients, several groups have used *in vivo* purging with rituximab in patients with NHL (Gisselbrecht, 2002). The technique of *in vivo* purging refers to the removal of circulating malignant B cells (in

the case of NHL) before stem cell harvest, generally by use rituximab combined with conventional chemotherapy (Jacobson et al, 2004). To assess the efficacy of rituximab as an *in vivo* purging agent, specifically in follicular lymphoma, the clearance of *bcl-2* gene has been used to monitor minimal residual disease (Gisselbracht, 2002) as detection of *bcl-2* positive cells in the peripheral blood post-ASCT has been associated with increased risk of lymphoma relapse (Freedman et al, 1999). In contrast to *in vitro* purging, around 80% of stem cell harvests after *in vivo* purging with rituximab have been *bcl-2* negative (Gisselbrecht, 2002). The use of rituximab as an *in vivo* purging agent does not adversely affect the number of stem cells harvested, stem cell function, and hematologic engraftment post-ASCT (Gisselbrecht, 2002; Jacobsen et al, 2004).

2. Pharmacological purging techniques

Pharmacologic purging is less selective than immunologic purging, and relies on the relative insensitivity of resting marrow stem cells to doses of cytotoxic drugs that are capable of eliminating clonogenic tumor cells. The most extensively investigated agents in this setting have been 4-hydroperoxycyclophosphamide (4HC) and mafosfamide (Jones et al, 1990; Gorin et al, 1991). Other agents that have been used include: ARA-C, mitoxantrone, epirubicin, paclitaxel, ifosfamide, carboplatin, etoposide, and cisplatin. Randomized studies are warranted to assess the role of chemotherapy based purging techniques on clinical outcomes after ASCT.

3. Photosensitizing purging agents

The relative sensitivity of tumor cells to light exposure after photosensitization with agents such as merocyanine 540 has been used for marrow purging (Atzpodien et al, 1987). The photosensitizers dibromorhodamine has been demonstrated to accumulate in malignant cells resulting in the eradication of 3 to 6 logs of NHL and chronic lymphocytic leukemia (CLL) cells with preservation of normal stem cells in the autograft (Roy et al, 2000).

4. Physical and membrane-active purging agents

Membrane-active agents such as alkyllysophospholipids have been investigated because of their relative selectivity for tumor cells (Okamoto et al, 1988). The antibiotic taurolidine has shown cytotoxic activity against a variety of leukemia and solid tumor cells (Ribizzi et al, 2002). Pulsed electric fields are used to increase the potential difference across the cell membranes of normal vs malignant cells. Therefore, threshold electric fields can be used to eliminate malignant cells, which are usually found in the large cell subsets, and spare the smaller stem cells (Eppich et al, 2000).

5. Molecular purging techniques

Molecular approaches for purging have largely been based on targeting of mutated or oncogene mRNA (Hirai et al, 2000) and critical proteins with short half-lives like c-myc (Luger et al, 2002). Antisense oligonucleotides

against bcr-abl mRNA have also been used in patients undergoing ASCT for chronic myelogenous leukemia (CML) (Szczylik et al, 1991, Leopold et al, 1995).

6. Immune effector cells purging

Cytotoxic immune effector cells, such as lymphokine-activated killer cells (LAK), have been shown to be effective in purging bone marrow contaminated with either a lymphoma cell line or autologous acute myeloid leukemia or acute lymphoblastic leukemia cells (Gambocorti-Passerini et al, 1991). Recently, the use of human derived natural killer (NK) cell line (NK-92) has shown efficacy as a purging agent to decrease or eliminate malignant clones in CML patients undergoing ASCT (Maki et al, 2003).

C. Clinical outcome of purged autografts

The clinical significance of tumor cell contamination of the autograft and purging remains controversial. Several studies suggest a survival benefit of purged autografts (Shimoni et al, 2002; Bierman et al, 2003), however others report no difference in survival (Blystad et al, 2004; Jacobsen et al, 2004). Recently, two well designed, randomized studies have shown no difference in survival between purged and unpurged autograft (Stewart et al, 2001; Schouten et al, 2003). Using CD34-selection purging technique, Stewart et al, (2001) demonstrated in MM patients a median overall survival in the CD34-selected group of 50 months versus not reached in the unselected group ($p = 0.78$). The median disease free survival was 100 versus 104 weeks ($p=0.82$) with 67% of patients in the CD34 selected group and 66% of patients in the unselected group relapsing. In the CUP trial, using immunomagnetic antibody purging, there was no discernible difference in overall survival ($p=0.43$) or progression-free survival ($p = 0.95$) between patients allocated a purged autograft or an unmanipulated graft (Schouten et al, 2003). If purging in ASCT is not beneficial, it is hard to argue that the infusion of minimal numbers of "undetectable" tumor cells from the stem cell graft could have a significant impact on the tumor relapse rate after ASCT.

IV. Immunologic engineering of the autograft

The anti-tumor activity of the immune system in the setting of hematopoietic stem cell transplantation has largely been described in the context of the GVT effect of Allo-SCT. We have recently reported a critical role of early post-ASCT lymphocyte recovery and its dramatic impact on clinical outcome. The highly significant relationship between autograft lymphocyte content, post-ASCT lymphocyte recovery and clinical outcome across a number of disease conditions, suggests that manipulations (engineering) of the autograft lymphocyte content may directly impact survival in ASCT (Porrata et al, 2004a).

A. Absolute lymphocyte recovery after stem cell transplantation

Early absolute lymphocyte count (ALC) recovery, as a surrogate marker of immune reconstitution after Allo-SCT has been associated with prolonged survival (Pavletic et al, 1998; Powles et al, 1988). To assess whether early ALC recovery has prognostic significance following ASCT, we analyzed ALC recovery at day 15 (ALC-15) post-ASCT in MM and NHL patients. The median overall survival (OS) and progression-free survival (PFS) in the MM group were significantly better for patients that achieved an ALC-15 ≥ 500 cells/ μl versus those that did not (33 months vs 12 months, $p<0.0001$; 16 months vs 8 months, $p<0.001$, respectively). For NHL patients the median OS and PFS were also significantly better for patients with ALC-15 ≥ 500 cells/ μl versus ALC-15 < 500 cells/ μl (not reached vs 6 months, $p<0.0001$; not reached vs 4 months, $p<0.001$, respectively) Multivariate analysis comparing ALC with all known prognostic factors in MM and NHL demonstrated that ALC-15 is a powerful, independent prognostic indicator for OS and PFS rates for both groups of patients (Porrata et al, 2001a). We've reported similar observations in patients undergoing ASCT for acute myelogenous leukemia (AML) (Porrata et al, 2002a), Hodgkin's lymphoma (Porrata et al, 2002b), metastatic breast cancer (Porrata et al, 2001b), and primary systemic amyloidosis (Porrata et al, 2005). These findings were confirmed by a number of investigators (Ferrandina et al, 2003; Gordan et al, 2003; Kim et al, 2004, Nieto et al, 2004). The superior survival observed based on ALC recovery post-ASCT suggests the existence of a possible autologous graft versus tumor effect (Porrata et al, 2004a).

B. Early post-ASCT immunological recovery

In contrast to the delayed quantitative and qualitative T- and B-cells reconstitution after ASCT (Guillaume et al, 1998; Porrata et al, 2001c), natural killer (NK) cells recover normal levels of function (and numbers) by 14 days after ASCT (Porrata et al, 2001d). Likewise, NK cells have been reported to be the first lymphocyte subset to recover after standard chemotherapy (Mackall et al, 1994), ASCT (Talmadge et al, 1997), and Allo-SCT (Kook et al, 1996), regardless of underlying disease. NK cell maturation can occur without a functional thymus in mice and humans, perhaps allowing for the prompt NK cell recovery in adults (Miller et al, 1992; Lotzova et al, 1993). Recently, we have shown in NHL patients that patients achieving normal absolute NK cell numbers by day 15 after ASCT have a superior median OS and PFS compared with patients with low absolute NK cell numbers by day 15 post-ASCT (not reached vs 26 months, $p< 0.0011$; not reached vs 6 months, $p< 0.001$, respectively). Even if ALC-15 is less than 500 cells/ μl , if the patients' NK cell counts have normalized, they have beneficial clinical outcome (Porrata et al, 2003a). Thus, it appears that NK cells may play an important immune surveillance role, as the only lymphocyte subset with

normal numbers and anti-tumor function in ALC-15 recovery early post-ASCT (Porrata et al, 2004a).

C. Stem cell autograft as a source of lymphocyte count post-ASCT

If CD34 positive selection purging technique is not used, the stem cell autograft has two possible sources affecting lymphocyte recovery post-ASCT: (1) the re-infused stem cells (CD34+ stem cells) and (2) the re-infused autograft lymphocytes (Porrata et al, 2004a). We reported that the autograft absolute lymphocyte count (A-ALC) collected in the stem cell autograft and re-infused to the patient not only correlated with ALC-15, but also with OS and PFS. We demonstrated superior OS and PFS in NHL (Porrata et al, 2004b) and MM (Porrata et al, 2004c) patients if they were infused with an A-ALC 0.5×10^9 lymphocytes/kg. Faster lymphocyte recovery post-ASCT was observed in patients infused with an A-ALC 0.5×10^9 lymphocytes/kg. The main lymphocyte subsets harvested during PBSC are T cell and NK cells (Verbik et al, 1995). We reported that the collected and infused autograft NK cells correlates with ALC-15 (Porrata et al, 2003b) and not T cells, B cells, or CD34 + stem cells. These data suggest that the stem cell autograft in ASCT could be used not only to harvest CD34+ stem cells for hematologic engraftment, but also immunocompetent effector cells (i.e. NK cells) to achieve a faster immunological engraftment post-ASCT. Thus, strategies to engineer an immunologic competent autograft will translate into significant improved clinical outcomes post-ASCT.

D. Immunologic graft engineering of the stem cell autograft

Strategic approaches to engineer an immunologic autograft in the ASCT have been very limited. The emphasis of immunologic therapy in the ASCT setting has been in the post-transplant period to target minimal residual including cellular or cytokine therapy (Benyunes et al, 1993; Lister et al, 1995; Hsu et al, 1996, Perillo et al, 2002, Laport et al, 2003). In addition to using cytokine (i.e. interleukin-2) to increase mobilization of immune effector cells into the autograft, other potential interventions to engineer an immunologic competent autograft by maximizing lymphocyte harvesting with direct impact on immunologic recovery and survival post-ASCT include: 1) the stem cell mobilization regimen, 2) the number of apheresis collections, and 3) adjustment of apheresis machine parameters.

1. Cytokines

Interleukin-2 has been used in combination with G-CSF to mobilize immune effector cells (i.e. NK cells) to harvest in the autograft and re-infused to the patient. In a small pilot study, using intent-to-treat analysis, Sosman et al (Sosman et al, 2001) found no statistical significance for PFS ($p = 0.5$) or OS ($p = 0.7$) favoring the 23 patients mobilized with G-CSF + IL-2 compared with the nine patients mobilized with G-CSF alone. Higher NK cell recovery by day 14 post-ASCT was found in the IL-2 +G-

CSF group. In a Phase I dose escalation study of IL-2 following G-CSF administration during stem cell collection, we identified a dose of IL-2 that maximizes NK cell autograft harvesting (Micallef et al, 2005). We are planning to proceed with a Phase II study using the target IL-2 dose to enhance NK cell autograft collection and its association with NK cell recovery and survival post-ASCT. The incubation of IL-2 in the autograft has been used to activate immune effector to target contaminated tumor cells in the autograft, in addition to infuse activated immune effector cells to target MRD in the host (Berkhan, 2000).

Other combinations of NK cell specific cytokines, such as interleukin-15 (Fehniger et al, 2001) and interleukin-21 (Parrish-Novak et al, 2000), with G-CSF could be studied to assess their impact on autograft NK cell collection, NK cell recovery post-ASCT, and clinical outcomes post-ASCT.

2. Stem cell mobilization regimens

The same concept of using a stem cell mobilization regimen to maximize the number of CD34+ stem cells mobilized from the bone marrow into the peripheral blood to obtain adequate numbers of stem cells for ASCT applies to lymphocyte harvesting. As ALC-15 depends on the amount of infused A-ALC, it is logical to assume that the collection of A-ALC will depend on the peripheral blood ALC at the time of collection (PC-ALC). We identified a positive correlation between PC-ALC and A-ALC (Porrata et al, 2004c, 2004d). Thus, any interventions that might result in pre-collection lymphopenia may negatively impact post-ASCT lymphocyte recovery and ultimately result in poor clinical outcomes. Preliminary evidence suggests that MM patients mobilized with the combination of growth factor (G-CSF) and chemotherapy (cyclophosphamide) had lower numbers of peripheral blood lymphocytes at the time of stem cell collection (low PC-ALC) leading to poor A-ALC collection compared to patients mobilized with growth factor alone (Porrata et al, 2004c). The clinical impact of this intervention is currently under investigation.

3. Number of apheresis collections

The number of apheresis collection depends on the stem cell mobilization regimens and the target dose of collected CD34+ stem cells. Patients mobilized with growth factor and chemotherapy tend to mobilize higher numbers of CD34+ stem cells from the bone marrow into the peripheral blood requiring less number of apheresis collections compared to patients mobilized with growth factor alone. Currently in most centers, the goal of apheresis is to collect the requisite stem cells in fewest collections possible. The number of apheresis collections as and its impact on collection of lymphocytes with possible impact on post-ASCT lymphocyte recovery and clinical outcome has not been studied until very recently. We demonstrated the increased number of apheresis collections correlated with higher harvested A-ALC translating into faster immune recovery and better survival post-ASCT. NHL patients that underwent 4 apheresis collections had superior OS and PFS compared to patients

with < 4 apheresis collections (86 months vs 18 months, $p < 0.0001$; 76 months vs 10 months, $p < 0.0001$, respectively) (Porrata et al, 2004d). Thus, efforts directed at decreasing total lymphocyte content of the autograft resulting in more efficient CD34 collection may have a negative impact on post-ASCT survival.

4. Apheresis machines

Apheresis machines in the ASCT setting have been viewed mainly as the instrument to harvest CD34+ stem cells. Three apheresis machines are used for stem cell collection in the ASCT setting including the COBE Spectra, the Fenwal CS 3000, and the Baxter Amicus. All these machines have similar CD34+ stem cell yield efficiency with the exception of the Baxter Amicus that in addition minimizes platelet consumption, therefore decreasing the rates of thrombocytopenia frequently observed during apheresis collections (Ford et al, 2002; Moog, 2002; Adorno et al, 2004). The role of these machines to harvest lymphocytes in addition to stem cells has not been studied. We performed an analysis of the ability of these machines to harvest lymphocytes on patients collected with the same machine. Preliminary data suggest that the COBE Spectra collected higher number of lymphocytes leading to a faster immunologic recovery and superior survival post-ASCT compared to the Fenwal CS

3000 or the Baxter Amicus (Katipamula et al, 2005). The survival benefit observed by the COBE Spectra was directly related to the higher number of harvested lymphocytes (A-ALC) that were subsequently re-infused to the patients. In comparison to the Fenwal CS 3000 or the Baxter Amicus, the COBE Spectra is able to collect higher numbers of mononuclear cells leading to a higher lymphocyte collection. This data suggest that apheresis instrument should be optimized to collect more lymphocytes (increased A-ALC) (Katipamulpa et al, 2005).

V. Conclusions

The development of ASCT for the treatment of hematologic malignancies has had a significant impact in the clinical outcomes of these patients. However, there is still room for improvement. The focus of this review has been a discussion of important new developments in autograft engineering as a means of improving clinical outcomes post ASCT. **Figure 1** summarizes strategies that may create the optimal autograft resulting in improved post-ASCT clinical end-points. The strategies can be divided into two categories. The first is to develop stem cell mobilization regimens to not only maximize CD34+

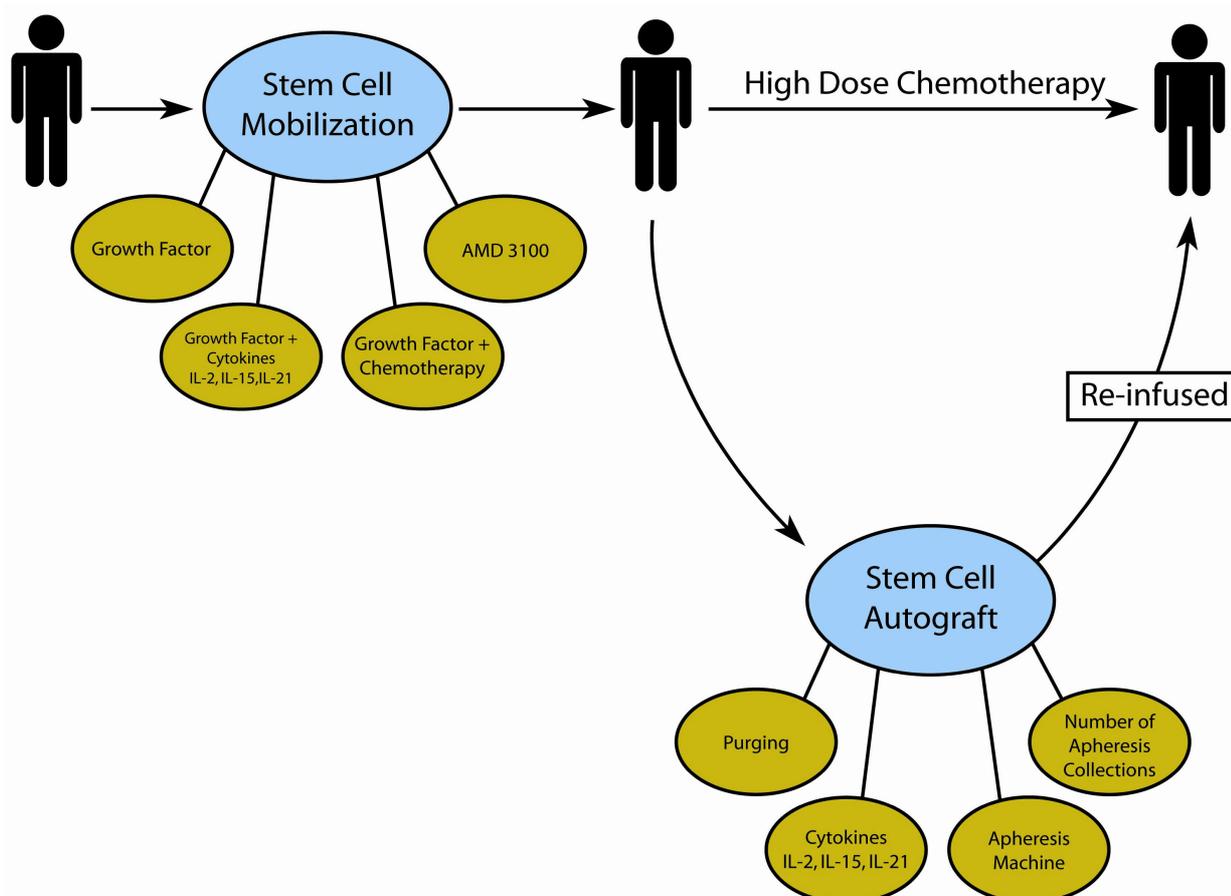


Figure 1. Schematic representation of immunologic graft engineering strategies in autologous stem cell transplantation targeting stem cell mobilization and stem cell autograft. Stem cell mobilization strategies include the use of stem cell mobilization regimens to enhance mobilization of immune effector cells into the peripheral blood for harvesting. Stem cell autograft strategies include manipulation of the stem cell autograft harvesting process by increasing number of apheresis collection, augmenting the capability of the apheresis machine to harvest lymphocytes, incubation of the autograft with cytokines to activate immune effectors cells, and immune effector cells purging techniques.

stem cells mobilization into the peripheral blood, but also immune effector cells (i.e. NK cells).

Considering the role of lymphocyte content in the autograft, maximizing lymphocyte content appears to be of great clinical significance. The second category is modification of the stem cell autograft collection process directed at minimizing tumor content and maximizing hematologic and immunologic reconstitution. We believe that rational strategies directed at optimizing the hematopoietic and immunologic characteristics of the autograft will significantly affect survival of patients undergoing ASCT as the autograft should not only be perceived as a means of hematological rescue following high-dose chemotherapy but also as an effective anti-tumor agent with significant potential for the treatment of malignant diseases.

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