Analysis of clinical outcomes in autologous stem cell transplant in relation to CD34+ cell count: A retrospective chart review

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Abstract

Background:

Autologous hematopoietic stem cell transplant (Auto-HSCT) is a well-established treatment for various hematologic malignancies, such as lymphoma and multiple myeloma (MM). Auto-HSCT requires stem cells to be collected from the patient themselves after undergoing a conditioning regimen. At our facility, the adequacy of the collected product is assessed through the measurement of CD34+ cells within a sample, as the product must be cryopreserved and subsequently thawed prior to re-infusion into the patient.

Rationale:

This study aims to determine if our current collection methods reflect an accurate measurement of the number of viable CD34+ cells collected within the product the patient receives. Additionally, we determine if a higher CD34+ cell count correlates with improved clinical outcomes such as time to neutrophil and platelet engraftment, treatment-related infection, intensive care unit (ICU) admission, and platelet transfusion.

Methodology:

This study was a retrospective chart review of patients who received Auto-HSCT at the Health Sciences Centre in St. John's, NL, between January 2014 and August 2019.

Results:

We found that post-thaw viable CD34+ cell count was positively correlated with time to neutrophil engraftment but not to time to platelet engraftment. There was no correlation between post-thaw viable CD34+ cell count and treatment-related infection, ICU admission or platelet transfusion.

Conclusion:

We did not find any evidence to support a correlation between post-thaw viable CD34+ cell count and clinical outcome; therefore, exploring other means of quality assurance for autologous stem cell transplant products at our institution may be beneficial.

Lay Summary

Many patients with certain types of cancers such as lymphoma and multiple myeloma (MM) need to receive a special type of stem cell transplant called an autologous stem cell transplant to treat their disease. The stem cell transplant takes place by collecting the patient's own blood, freezing it, then thawing it weeks later and re-infusing it into the patient after they have received some other treatments.

At the Health Sciences Centre in St. John's, NL, we measure the amount of a certain type of cell called a CD34+ cell from a blood sample collected from the patient. We then use this number to determine the quality of the product, with higher numbers of cells thought to be correlated to better products and therefore better patient outcomes. The process of measuring these cells is labour and cost-intensive. We would therefore like to determine if this cell number is correlated to superior patient outcomes, or if we should look for new and better ways to determine the quality of the product.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AGIHO	Infectious Diseases Working Party
Allo-HSCT	Allogeneic Hematopoietic Stem Cell Transplant
ANC	Absolute Neutrophil Count
ASCO	American Society of Clinical Oncology
Auto-HSCT	Autologous Hematopoietic Stem Cell Transplant
BEAM	Carmustine, Etoposide, Cytarabine, and Melphelan
BuMelTT	Busulfan, Melphelan, and Thiotepa
CCI	Charlson Comorbidity Index
CD	Cluster of Differentiation
CFU-GM	Colony-Forming Units-Granulocytes/Monocytes
CHOP	Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone
CMV	Cytomegalovirus
CNS	Central Nervous System
CR	Complete Remission
CRD	Cyclophosphamide, Lenalidomide, and Dexamethasone
CRF	Controlled-Rate Freezing
CRu	Complete Remission unconfirmed
CR1	Clinical Remission One (first clinical remission)

CT	Computerized Tomography
CXCL12	CXC Motif Chemokine Ligand 12
CXCR4	CXC Chemokine-Related Receptor 4
CyBorD	Cyclophosphamide, Bortezomib, and Dexamethasone
Dara-VTd	Daratumumab, Bortezomib, Thalidomide, and Dexamethasone
DGHO	German Society for Hematology and Medical Oncology
DLCBL	Diffuse Large B-Cell Lymphoma
DMSO	Dimethyl sulfoxide
DRd	Daratumumab, Lenalidomide, and Dexamethasone
EBMT	European Group for Blood & Marrow Transplantation
ECOG	Eastern Cooperative Oncology Group
FDG	Fluorodeoxyglucose
G-CSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
GVT	Graft-Versus-Tumour
GVHD	Graft-Versus-Host Disease
HCT-CI	HCT-Comorbidity Index
HHV	Human herpesvirus
HIV	Human Immunodeficiency Virus

HLA	Human Leukocyte Antigen
HL	Hodgkin Lymphoma
HSCT	Hematopoietic Stem Cell Transplant
HSV	Herpes Simplex Virus
HTLV	Human Lymphotropic Virus
HyperCVAD	Hyperfractionated Cyclophosphamide, Vincristine, Doxorubicin, and Dexamethasone
ICU	Intensive Care Unit
KPS	Karnofsky Performance Score
MDR	Multidrug-resistant
MEAM	Ranimustin, Etoposide, and Melphalan
MM	Multiple Myeloma
MRD	Minimal Residual Disease
NCC	Nucleated Cell Count
NHL	Non-Hodgkin Lymphoma
PBPC	Peripheral Blood Progenitor Cell
PAM	Pretransplant Assessment of Mortality
PCR	Polymerase Chain Reaction
PET/CT	Positron Emission Tomography/ Computerized Tomography

PTH	Parathyroid Hormone
R-CHOP	Rituximab, cyclophosphamide, Doxorubicin, Vincristine, and Prednisone
R-DHAP	Rituximab, Dexamethasone, Cytarabine, and Cisplatin
SFPR	Secondary Failure of Platelet Recovery
TBI	Total Body Irradiation
TNC	Total Nucleated Cell
TRM	Transplant-Related Mortality
UCF	Uncontrolled-Rate Freezing
VRd	Bortezomib, Lenalidomide, and Dexamethasone
VTd	Bortezomib, Thalidomide, and Dexamethasone
VZV	Varicella Zoster Virus
WHO	World Health Organization

Chapter 1: Background and Rationale

1.1 Curative Potential of Hematopoietic Stem Cell Transplant

Hematopoietic stem cell transplant (HSCT) is a means of treatment for a variety of hematologic and non-hematologic malignancies, and non-malignant conditions. While several different treatments either slow the progression of or offer symptomatic relief of hematologic malignancies, few treatments provide the curative potential of HSCT.[1] HSCT involves the harvesting of stem cells which are most commonly extracted from the peripheral blood, though they may also be taken from the umbilical cord or extracted from the bone marrow itself. The cells are subsequently infused into the patient through an intravenous line with the goal of engraftment into the patient's bone marrow. The cells may be harvested from the patient themselves in the setting of autologous HSCT (auto-HSCT), or from a donor in the setting of allogeneic HSCT (allo-HSCT). In the setting of auto-HSCT, the primary treatment lies in eradicating the malignant cells through chemotherapy, while the subsequent transplant acts as a hematopoietic rescue. Once the cells engraft, they can take on the hematopoietic function necessary for the individual's survival, providing a reservoir of stem cells with which to replenish all of the blood-containing tissues of the body.[2]

While the reward of curative treatment is high, HSCT also poses a significant risk to patients. Prior to infusion of either donor (for allo-HSCT) or the patient's own stem cells (for auto-HSCT), the patient must undergo a conditioning regimen to ablate their current hematopoietic stem cells. In the case of allo-HSCT this ablates their native cells to allow the infused stem cells to engraft, while in auto-HSCT, the goal is to reconstitute hematopoietic function after eradicating the malignant cells occupying the bone marrow. These conditioning regimens

inevitably and purposefully lead to a period of profound pancytopenia prior to engraftment and restoration of hematopoietic function of the newly infused stem cells. Pancytopenic patients are deficient in blood cells of all lineages leaving them highly susceptible to a variety of complications including anemia, bleeding and infection.[3]

1.2 Hematopoiesis

Hematopoiesis involves the generation and proliferation of multippotent progenitor cells which ultimately commit to a particular lineage of blood cell, either myeloid or lymphoid, which will subsequently differentiate into more mature hematopoietic and immunologic cells such as erythrocytes, megakaryocytes, granulocytes, and B- and T-cells, among others. Along with their differentiation capabilities, stem cells are capable of self-renewal which creates a steady state of cellularity from which tissues can draw should they acquire damage. A single stem cell will produce approximately 1,000,000 mature cells after twenty cell divisions; however, these cells can also respond to hematopoietic growth factors to increase production to even greater numbers during times of stress.[4]

Hematopoiesis begins on approximately the 16th day of gestation in the embryonic yolk sac, though limited to the production of platelets and red blood cells at this point to support the newly developed circulatory system by facilitating oxygen transport, as well as monocytes which will ultimately develop into immune cells in the brain (microglial cells) and liver (Kupffer cells). Hematopoietic stem cells make a second appearance around three to four weeks of gestation in the aorta-gonads-mesonephros region. Stem cells from this region, in conjunction with stem cells from the yolk sac migrate through the blood, and ultimately infiltrate the liver, spleen and bone

marrow. The liver and spleen are the principal source of hematopoietic cells for the fetus after 6 weeks gestation until about five to seven months of gestation, though these organs continue to produce cells until two weeks after birth. Around six weeks of gestation, the placenta and umbilical cord also become a source of hematopoietic progenitor cells for the growing fetus and continue to function until birth. By seven to eight weeks of gestation, progenitor cells from the liver begin to seed the developing thymus, which will ultimately function as a major site for lymphocyte production, maturation, differentiation and selection.[4,5]

At about five to seven months of gestation, the fetal bone marrow takes over as the most important site for hematopoiesis. In infants, the marrow of almost all bones is involved in hematopoietic cell production, but in adults, hematopoiesis is typically confined to the marrow of the vertebrae, ribs, sternum, skull, sacrum and iliac bones of the pelvis, and proximal ends of the long bones, particularly the humeri and femurs.[4]

Hematopoietic cells are produced continuously throughout an individual's lifetime. They coexist at various stages of maturation principally within the bone marrow. However, hematopoiesis may also occur through extramedullary tissues such as the liver, spleen and lymph nodes in some circumstances of hematopoietic stress, such as anemia.[3,6] The use of extramedullary sources for increased hematopoietic function can be beneficial and function as a physiologic compensatory mechanism at times of hematopoietic stress; however, extramedullary hematopoiesis can also be seen in pathological conditions, such as chronic myeloid leukemia which tends to manifest with hepatosplenomegaly and lymphadenopathy.[5]

All hematopoietic cells in the body have ultimately arisen from a hematopoietic progenitor stem cell, which stands at the top of the hematopoietic hierarchy. They are multipotent cells, defined as cells that have the potential to differentiate and give rise to all other types of hematopoietic cells. To ensure that the stem cell population maintains its ability for self-renewal, it is thought that each time a stem cell divides, on average, at least one of the two daughter cells produced will remain undifferentiated as a stem cell to become part of the stem cell reservoir from which the body can draw to replenish any type of hematopoietic cell that is needed. There are, however, exceptions to this statement and symmetric divisions do occur. For example, a hematopoietic stem cell in the liver of a fetus may undergo a symmetric division in which both daughter cells remain undifferentiated to build the reservoir of stem cells from which the rapidly developing fetus may draw. Conversely, at times of hematopoietic stress, an individual's stem cells may symmetrically divide into two differentiated cells through a process called *commitment*, to expedite the process of tissue regeneration where it is needed.[5]

The dynamic nature of hematopoiesis allows the body to rapidly address hemopoietic challenges as they arise. Over a typical day, an individual's marrow produces approximately 200,000,000,000 erythrocytes, 100,000,000,000 platelets and 60,000,000,000 neutrophils. This production rate is sufficient to match the rate of peripheral destruction of cells already in circulation; however, should states of anemia, thrombocytopenia, or neutropenia arise, the hematopoietic system can, in most individuals, adjust to meet the extra production demand. Conversely, an individual may also experience secondary erythrocytosis, thrombocytosis, or granulocytosis in such situations as inflammation. At these times, the hematopoietic system can

also slow its production, as the overproduction of these cell lines can have potentially devastating consequences, such as thrombosis.[5,7]

Rather than continuously dividing at a fixed rate, the hematopoietic stem cells within the bone marrow spend most of the time in the *quiescent* phase, characterized by a resting state. At other times, approximately every few months, the cells "awaken" and divide. It is thought that the quiescent phase may help the stem cells maintain their multipotent abilities and protect against the acquisition of mutations that can lead to transformation of the cells and subsequently, malignant processes.[8] Some stem cells do exist in the peripheral circulation, yet they possess a unique homing ability that allows these circulating cells to migrate through the capillaries within the bone marrow in areas that have high levels of CXC motif chemokine 12 ligand (CXCL12). The cells then congregate around blood vessels in the interstitium to interact with their mesenchymal, endothelial and neural cells to create a specialized microenvironment hospitable to stem cells called a *niche*. The cells in the niche environments release certain factors, including CXCL12 that seem to regulate stem cell behaviour, promoting quiescence.[5,9] The mechanism is incompletely understood, yet it is thought that the stem cell population may only be stimulated to divide and multiply in the presence of increased growth factors and decreased quiescent factors. Another theory states that stem cells may be stimulated to produce progeny depending on relative vacancy within bone marrow niche areas, though extramedullary niches may also be created during hematopoietic stress.[5]

For simplicity, we generally explain the process of hematopoiesis as a progenitor cell committing to either lymphoid or myeloid lineage. In reality, this process is more complicated; there are

intermediate steps within the differentiation process at which time some progenitor cells may differentiate into either a lymphoid cell, or a narrow spectrum of myeloid cells, but subsequent cell divisions will only give rise to a single type of cell. Cells of myeloid lineage ultimately produce erythrocytes, granulocytes, monocytes and megakaryocytes while cells of lymphoid origin differentiate into B-cells, T-cells and natural killer cells, as seen in Figure 1.1.[10]

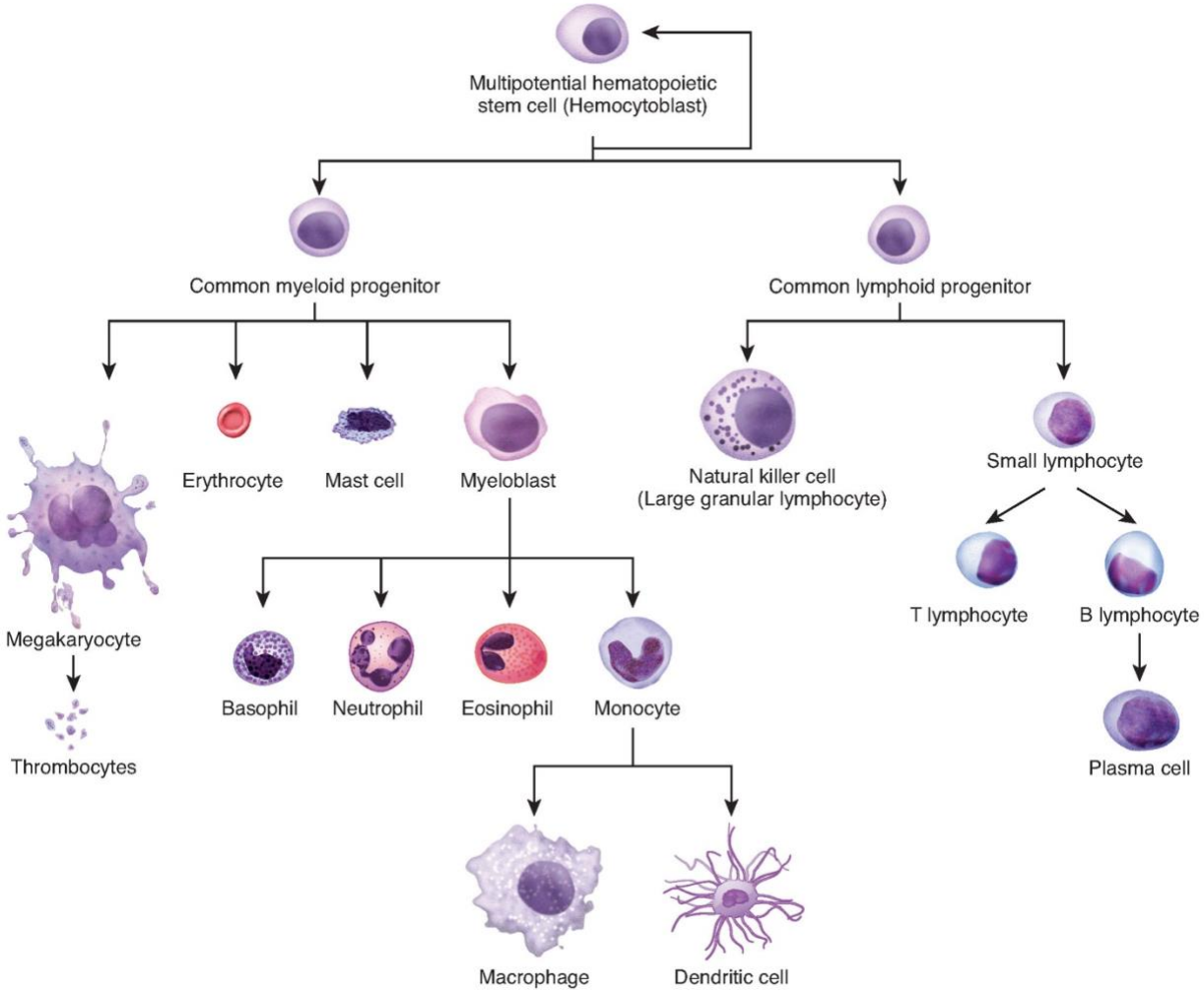

Figure 1.1 Hierarchy of Hematopoietic Progenitor Cells. Adapted from open access source:

Hematopoiesis. Textbook OpenStax Anatomy and Physiology, Version 8.25,[11]

1.3 History of Hematopoietic Stem Cell Transplantation

The earliest attempts at HSCT took place in the 1950s. The tremendous number of fatalities from the atom bomb that struck Japan in 1945 prompted scientists to investigate ways to protect humans from irradiation. Jacobson et al. first applied this concept in mice in 1949 by observing that the animals could survive a lethal dose of radiation by exteriorizing the spleen to protect it from being irradiated.[12,13] Lorenz et al. subsequently discovered that the same improved survival could be achieved by infusing splenic cells into the bone marrow of the mice in 1951.[13,14] Throughout the 1950s, experimentation demonstrated engraftment of donor-derived bone marrow cells could be seen in lethally irradiated mice and dogs.[13,15,16]

The concept was first applied to humans in 1957 by Thomas et al. when patients with acute leukemia were irradiated and treated with chemotherapy to eliminate the leukemia cells. These patients were subsequently infused with bone marrow cells from healthy donors.[13,17,18] Of the six patients transplanted, only two patients were shown to have transient hematopoietic engraftment of donor cells, and unfortunately, none of the patients survived beyond 100 days post donor cell infusion. In 1959, the same group of researchers published a description of a patient with what was determined to be terminal leukemia who received total body irradiation (TBI) to eradicate their marrow and was subsequently infused with bone marrow cells extracted from the patient's identical twin. The patient experienced swift recovery of hematopoietic function and the progression of the leukemia was halted for four months. This was a key moment in the evolution of hematopoietic stem cell transplantation as it was the first confirmation in humans that complete radiation and destruction of the marrow of a patient with leukemia followed by infusion of bone marrow cells from a well-matched donor could demonstrate anti-leukemic

properties.[19] Also in 1959, after six people, unfortunately, were exposed to a potentially lethal dose of radiation from a failing reactor in Belgrade, Serbia, Mathé et al. infused these six patients with donor marrow. Though five of these patients did survive, there was no definitive evidence demonstrating donor marrow engraftment restoring hematopoietic function to the patient, so the contribution of transplant to the survival of these patients was unclear.[2]

The earliest attempts at auto-HSCT took place a year prior, in 1958. Kurnick et al. described two cases of patients with metastatic malignancies whose marrow was collected and frozen. The patients underwent extensive radiation therapy, after which the collected marrow was thawed and re-infused into the same patients. While one of these patients died, the second demonstrated hematopoietic recovery after a long period of pancytopenia.[20] The first evidence of long-term success with auto-HSCT was seen when a 21-year-old woman living in Pennsylvania was admitted to hospital in 1959 and soon diagnosed with follicular lymphoma. The patient's bone marrow was aspirated and subsequently frozen prior to the initiation of chemotherapy (high-dose mustard-conditioning regimen). Approximately one week after collection, upon evidence that the patient's marrow was completely aplastic, the bone marrow cells were reinfused. She experienced a period of pancytopenia for approximately 15 days post bone marrow cell infusion, after which her laboratory investigations demonstrated signs of restoration of hematopoietic function. Granulocytes, followed by platelets, then reticulocytes recovered, in that order and within a few days of each other. This patient lived for 30 years post-transplantation, the majority of the time in complete remission from her lymphoma.[21]

While these early studies provided a framework on which to build, outcomes of early studies tended to be poor with significant mortality. This can be attributed to the lack of understanding of conditioning regimens, histocompatibility matching and graft-versus-host disease (GVHD) at the time. Many researchers abandoned the field, discouraged by the initial difficulties, but through further animal studies, scientists gained valuable insights which they were able to apply to the field of human HSCT in order to obtain more successful outcomes.[9] In 1968, through their work with monkeys, Van Putten et al. developed a HSCT transplant protocol which led to the successful transplant of several human patients with immune deficiencies in the United States and the Netherlands using human leukocyte antigen (HLA)-identical sibling donors.[22,23] Van Rood et al. also contributed to this body of knowledge concerning the role of HLA in HSCT through their work with pregnant women, noticing that these patients formed antibodies against HLA types in the fetus of paternal origin which led to a greater understanding of the importance of HLA typing and matching in the selection of HSCT donors.[24,25]

1.4 Regulators of Hematopoietic Stem Cell Engraftment

After a stem cell transplant, restoration of hematopoietic function requires the successful interplay between the infused cells and the supporting microenvironment within the recipient's bone marrow. The infused stem cells must first adhere to the bone marrow endothelium. Next, stem cells migrate through trans-endothelial movement and intraparenchymal homing mechanisms to settle into their hematopoietic niches within the inner endosteal surface of the bones. This process is regulated mainly by a gradient created by the stromal cell-derived factor-1 (also known as CXCL12, as discussed in the previous section "Hematopoiesis") binding to CXC chemokine-related receptor 4 (CXCR4) within the extracellular matrix around hematopoietic

stem cells.[26] Studies have shown that CXCR4 expression on the surface of the hematopoietic stem cell membrane is necessary for appropriate homing and subsequent engraftment.[27] This concept was further explored when CXCR4 antagonists, such as plerixafor, were developed which helps to mobilize stem cells prior to collection.[2,28]

Bone marrow niches are microenvironments containing self-renewing mesenchymal stromal cells, regulatory T-cells and osteoblasts displaying parathyroid hormone (PTH) receptors.[29,30] The mesenchymal stromal cells promote engraftment of hematopoietic stem cells and therefore increase the efficacy of transplant if they are transplanted alongside the stem cells, according to a 2009 study on monkeys undertaken by Masuda et al.[31] Engraftment is also aided by osteoblasts which work with sinusoidal endothelial cells to produce several types of small molecules such as annexin II, vascular cell adhesion protein 1, intracellular adhesion molecule-1, cluster of differentiation (CD) 44, CD164 and osteopontin, each of which promote engraftment of hematopoietic stem cells into the bone marrow.[32,33] Animal studies have further demonstrated the importance of osteoblasts, when stimulated by PTH, in the proliferation and mobilization of stem cells, though the benefit in humans is questionable, as a study of the same molecules in humans using hematopoietic stem cells extracted from cord blood did not yield the same results.[34,35] Beyond these pathways, engraftment is also regulated by intrinsic genetic programming which governs states of quiescence, self-renewal and proliferation, differentiation into more mature cells, as well as apoptosis through cell interactions with marrow involved T-cells, adipocytes and fibroblasts.[2]

1.5 Sources of Hematopoietic Stem Cells

1.5.1 Bone Marrow

The popular term "bone marrow transplant" is still used, as, historically, stem cells were harvested directly from the bone marrow for transplantation, and in certain situations, may still be harvested directly from the bone marrow today. The hematopoietic stem cells are obtained by aspirating the bone marrow through a large bore needle. The most common extraction site is the posterior iliac crest on either side of the body obtained under local or general anesthesia. The cell dose generally considered adequate to enable long-term engraftment is 2×10^8 nucleated cells per kg of body weight of the recipient. Collections of ≤20 mL/kg of donor body weight are generally considered safe for the donor. Bone marrow extraction is a very safe and routine procedure that rarely leads to complications, and even then, most are self-limited.[2]

1.5.2 Umbilical Cord

In 1986, the first successful transplantation of stem cells harvested from unrelated umbilical cord blood took place, providing an alternative to direct bone marrow harvest.[9] Cells are collected directly from the blood vessels within the umbilical cord of the placenta at the time of delivery. One advantage of cord blood is that cells are immunologically naïve, as a newborn infant has yet to encounter significant external exposures to which they may develop antibodies, nor are they able to make antibodies at the time of their birth. This extends the spectrum of histocompatibility among recipients and may perhaps allow for allogeneic transplants for patients for which an HLA-matched adult donor may be difficult to find.[2,36] Since the induction of the use of cord blood in stem cell transplant in the late 1980s, over 20,000 allogeneic transplants have been completed using donor cells extracted from umbilical cord blood as of the year 2011.[37] While

cord blood provides an excellent alternative to bone marrow extraction for difficult to match recipients, a major limitation lies in the relatively small number of hematopoietic stem cells that can be extracted from units of cord blood relative to the mostly adult recipients who require a transplant, and most transplants will require two units of cord blood.[38]

To quantify the number of stem cells, which is used as a measure of adequacy for the transplant, we measure CD34+ cell count by flow cytometry. Stem cells express markers on the surface of their cell membranes which help to distinguish them from other types of cells. One of these surface markers is CD34.[5] Generally, the acceptable threshold for transplantation when using cord blood is $\geq 2.5 \times 10^7$ total nucleated cells or $\geq 2 \times 10^6$ CD34+ cells/kg of recipient body weight.[39,40] Difficulties may also arise when using CD34+ cell number as a measure of adequacy as some units have shown to have unexpectedly large discrepancies between CD34+ cell count and total nucleated cell count.[41]

1.5.3 Peripheral Blood

The first harvest of stem cells from the peripheral blood for transplant was in 1994. In recent times, 70-95% of all stem cell transplants are harvested from the peripheral blood. This method is now the preferred means for collecting stem cells as it is less invasive for the donor and tends to lead to faster engraftment, potentially leading to improved patient outcomes.[42] Randomized controlled trials have shown that transplants performed using peripherally harvested stem cells have a tendency to engraft more rapidly, provide a better quality of life and are lower in cost when compared to stem cell transplants harvested directly from bone marrow.[43–45] This procedure is also safer than direct marrow harvest, demonstrating only a 0.56% risk of adverse

events in a study of 7000 healthy donors, with the most common complication being self-limited bone pain after granulocyte colony-stimulating factor (G-CSF) administration.[46]

The stem cells taken from peripheral blood are harvested through a venous cannula and taken into a cell-separator machine. In the machine, mononuclear cells are separated and collected by centrifugation, after which the erythrocytes are returned to the donor. This process continues until an adequate number of mononuclear cells are collected. It is generally difficult to obtain an adequate supply of hematopoietic stem cells through untreated peripheral blood alone since stem cells are present only in very low numbers in the peripheral blood. In order to increase the number of stem cells in circulation, growth factors such as G-CSF can be administered, which can increase the number of circulating cells 10- to 100-fold.[4] G-CSF functions by inducing hyperplasia of the myeloid cells and promoting the release of CD34+ cells into the peripheral circulation through the cleavage of adhesion molecules.[47] Other agents used to mobilize stem cells within the peripheral blood are granulocyte-macrophage colony-stimulating factors (GM-CSF), interleukin 3 (IL-3), thrombopoietin and CXCR4 antagonists such as plerixafor.[2,28,48,49] Adequacy for transplantation is generally determined through CD34+ cell count with an acceptable threshold being >2 x 10^6 CD34+ cells/kg of recipient body weight.
If the number of CD34+ cells is insufficient for transplantation, a second collection is planned. At this time, another dose of G-CSF is administered and other agents, such as Plerixafor, a CXCR4 agent, can be administered in order to boost the CD34+ cell count. [9]

Once cells are collected by apheresis, defined as "the collection of blood from a patient or donor, followed by separation and removal of a cellular component(s) and /or plasma and return of the

remaining blood components to the patient or donor,"[50] the resultant product is called *peripheral blood progenitor cells* (PBPCs). In contrast to the term *blood stem cells* which refers to the hematopoietic stem cells themselves once they have been isolated from this product.[2]

1.6 Types of Hematopoietic Stem Cell Transplant

HSCT can be classified as autologous or allogeneic. The principle of HSCT in either form is to eradicate the patient's own hematopoietic and immune systems through chemotherapy and/or radiation therapy and to replace it with previously harvested stem cells which will engraft, proliferate and differentiate to re-establish a patient's ability to generate their own hematopoietic and immune cells.[4]

Many factors are considered when selecting the type of transplant a patient with a hematologic malignancy will receive including the age of the patient, the type of malignancy, the potential availability of a suitable donor, the potential, as well disease-specific factors such as the extent of bone marrow involvement, and the potential for collection of a disease-free autograft, sensitivity to chemotherapy, as well as the overall bulk of the disease.[51]

1.6.1 Allogeneic Stem Cell Transplant

The main mechanism of action in the setting of allo-HSCT is cancer eradication through immunologic recognition of residual tumour cells native to the host and their subsequent destruction by immune cells from the donor. This is referred to as graft-versus-tumour (GVT) effect. Allo-HSCT is the treatment of choice for many acute leukemias. Allo-HSCT involves harvesting stem cells from a donor. The cells are subsequently infused into the recipient who has

undergone some type of conditioning treatment to eradicate their own cells. Allo-HSCT offers an immune response against the malignant cells from the donor cells which allows for the patient to be potentially cured of their malignancy.[1] In comparison to auto-HSCT, allo-HSCT is accompanied by a greater risk of complications including a significantly greater chance of transplant-related mortality (TRM), and requires immunosuppression post-transplant to allow for engraftment and prevent GVHD, but puts the patient at higher risk of infectious complications. It also requires that a compatible donor be available to provide stem cells to restore hematopoietic function. It is also recommended that psychosocial resources be readily available to patients in order to help them cope with this highly time-consuming and difficult procedure.[2]

1.6.2 Autologous Stem Cell Transplant

Auto-HSCT is generally indicated for patients who will need aggressive chemotherapy, resulting in near total or total marrow ablation in order to eliminate the malignant cells, but have healthy marrows which will be able to restore hematopoietic function.[52] It has become the mainstay of treatment for patients with MM as well as aggressive lymphomas. It is more accessible to patients as no HLA-matched donor needs to be found, and often better tolerated with a lower risk of life-threatening complications overall and graft failure is rare. This type of transplant typically results in faster engraftment and a lower risk of opportunistic infections.[51]

Auto-HSCT is accomplished through the harvesting of the patient's own stem cells which are subsequently reinfused into the patient after undergoing a course of high-dose chemotherapy, allowing for hematopoietic reconstitution.[1] In the setting of an auto-HSCT, the patient must also receive chemotherapy prior to stem cell collection to eliminate malignant cells. Collection

normally takes place during the recovery phase of a cycle of chemotherapy after cell counts start to rise again.[4]

In the setting of most hematologic malignancies, the response to treatment is directly related to the dose of chemo-radiotherapy with a steeply rising dose-response curve demonstrating that the higher the dose, the higher the curative potential. Auto-HSCT has a relatively low transplant-related mortality rate. The fact that we typically now use PBPCs that have been mobilized rather than harvesting stem cells from the bone marrow has led to a shorter period of neutropenia in patients, ultimately reducing the period during which the individual is particularly susceptible to infection. This, in conjunction with improved supportive care measures contributes to a reduction in transplant-related mortality in these patients.[2]

1.7 Indications for Autologous Stem Cell Transplant

Auto-HSCT is generally indicated for patients with malignant disease that demonstrates respond readily to high-doses of chemotherapy. This would include diseases such as lymphoma, multiple myeloma (MM), germ cell tumours, and some pediatric tumours, such as neuroblastoma.[53,54] Extensive studies have investigated the role of auto-HSCT in breast and ovarian carcinoma as well as more limited studies in the setting of renal cell carcinoma and non-small cell and small cell lung cancer, but these studies have failed to demonstrate a consistent benefit with transplant.[55–61] Auto-HSCT functions through the use of high-dose cytotoxic therapy, followed by an autograft which acts as a rescue mechanism for the hematopoietic system, in contrast to allo-HSCT which is used for malignancies with extensive bone marrow involvement.[2]

1.8 Lymphoma

Lymphoma is the overarching title for a heterogenous group of malignant disorders that affect a specific type of hematopoietic cell, the lymphocyte. In comparison to other lymphocytes, lymphocytes progressing to malignant disease will acquire certain mutations that enhance their ability to grow and survive. In the setting of lymphoma, the malignant cells typically originate in lymph nodes or extra-nodal lymphatic tissue and may be localized or widespread at the time of diagnosis. The type of cell involved in malignant transformation is variable; it may be the B-cell, T-cell, or occasionally, the natural killer cell, though all are derivates of the lymphocyte lineage.

Lymphoma is a common diagnosis with approximately 77,000 new cases diagnosed in the United States in 2020 and represents 4.3% of all cancer diagnoses in the country. It is more common in men than women, as well as in those of European and Hispanic ethnicities. In general, there is an exponential increase in the risk of disease, particularly non-Hodgkin Lymphoma (NHL), with increasing age.[62] Several potential occupational hazards have been identified, such as pesticides, herbicides, dyes, engine exhaust, and solvents, though the evidence that these exposures may contribute to the development of lymphoma is weak and inconsistent.[63–65] Risk of lymphoma is also slightly higher in individuals with past radiation exposure, first identified through an increased incidence of lymphoma reported in survivors of the atomic bombings in Hiroshima and Nagasaki,[66] and also observed in survivors of the Chernobyl disaster.[67] Several infectious agents have been identified as potential pathogens with some evidence to suggest increased infectious risk with human T-cell leukemia/lymphoma virus-1, Epstein-Barr virus (EBV), human herpesvirus (HHV)-8, hepatitis B and C, *Helicobacter pylori* and *Chlamydophila psittaci*. Immunodeficiency has also been shown to be a risk factor in

the development of lymphoma, though it is difficult to accurately quantify this risk given the rarity of inherited immunodeficiency syndromes. Finally, several autoimmune diseases have also been found to be associated with an increased risk of lymphoma, thought to be secondary to chronic immune stimulation that promotes excessive B-cell proliferation and depressed regulatory T-cell function.[65]

While classification methods vary and have evolved greatly over time, the most widely accepted classification for lymphoma at the moment is the Ann Arbor Staging Classification system, which was originally established for the classification of Hodgkin lymphoma (HL), but later expanded to incorporate NHL as well.[68] In this classification system, the disease is divided into 4 stages, each subdivided and assigned either a 'B', should constitutional symptoms be present, such as a temperature >38.3ºC, weight loss of >10% of an individual's total body weight, or drenching night sweats, or are assigned an 'A' rating in the absence of such symptoms. Stage I describes a malignancy involving a single lymph node region or lymphatic structure, while stage II describes the involvement of at least two lymph node regions or structures on the same side of the diaphragm. If the malignancy has spread to involve lymph nodes or lymphatic structures on both sides of the diaphragm, the lymphoma is classified as stage III, while diffuse or disseminated involvement of one or more extra-lymphatic organs with or without associated lymph node involvement is classified as stage IV.[69]

Later, the Cotswold modification was added to the classification system in order to incorporate computerized tomography (CT) evidence of disease into staging categories, especially to identify bulky disease, which is termed 'X', or complete remission unconfirmed (CRu), in which case a

patient does have a residual mass seen on imaging after treatment, but this is favoured to represent scar tissue rather than actual residual disease.[70] In 2007, with the introduction of positron emission tomography/computerized tomography (PET/CT), the term 'CRu' became obsolete, as tissue could be much more accurately differentiated between disease and fibrous tissue using metabolic activity as a guide.[65,71] Several other classification systems for lymphoid neoplasms exist, 1 of the most well-known being the World Health Organization (WHO) classification of Tumours, first published in 2001,[72] with subsequent revisions in 2008 and 2016. This highly detailed document reflects consensus among hematologists, hematopathologists and geneticists.[73]

Another broad but important classification is HL versus NHL. HL is a malignancy characterized by neoplastic mature B lymphocytes. It represents approximately 10% of all lymphoma diagnoses and can itself be subdivided into classical HL, representing the majority of cases, and nodular lymphocyte predominant HL.[74] NHL encompasses a group of malignant disorders that are characterized by neoplastic proliferation of mature B-cells, T-cells, or natural killer cells. NHL can typically be distinguished from HL based on the absence of Reed-Sternberg cells in the former, though Reed-Sternberg cells can occasionally be found in some subtypes of HL. Within each cell type in NHL, diseases are subcategorized by their growth rate; slow-growing malignancies are labeled as 'indolent' lymphomas, such as follicular and marginal zone lymphomas, while faster-growing cancers are known as aggressive lymphomas, such as diffuse large B-cell or Burkitt lymphoma.[75]

1.8.1 Treatment of Lymphoma

The treatment of HL differs from NHL in part because approximately 80-85% of patients with HL will be cured through treatment with chemotherapy alone or sometimes in conjunction with radiotherapy, whereas the prognosis and treatment course tend to be more variable in the case of NHL. With a long list of subtypes under the heading of NHL, treatments vary widely as well. For example, follicular lymphoma may simply be observed, initially, or perhaps treated with radiation, though chemotherapy similar to that for diffuse large B-cell lymphoma (DLCBL) will likely be required further along in the disease course.[76]

Chemotherapy remains first-line treatment for patients diagnosed with aggressive B-cell lymphomas. Approximately 50% of patients with NHL achieve complete remission (CR) with current therapies.[77] In patients with relapsed or refractory aggressive NHL, there is a potential for a cure through salvage (second-line) chemotherapy, a high-dose regimen of chemotherapy for consolidation (chemotherapy administered after initial remission) , followed by auto-HSCT.[78,79]

1.8.2 Evidence for Autologous Stem Cell Transplant in Lymphoma

Auto-HSCT, in combination with high-dose chemotherapy, is typically considered the treatment of choice in relapsed or refractory aggressive B-cell lymphomas such as DLBCL. Allo-HSCT plays a much more limited role in the treatment of these conditions. Philip et al. established auto-HSCT with high-dose chemotherapy as superior to conventional chemotherapy alone in patients with relapsed chemotherapy-sensitive NHL by demonstrating an increased rate of event-free and overall survival.[1,78] Only patients with chemo-sensitive disease will derive benefit from auto-HSCT, and those with negative fluorodeoxyglucose (FDG)-PET prior to transplant demonstrate

significantly improved outcomes over those with residual disease detectable on FDG-PET.[80–82] While historically, auto-HSCT was able to achieve long-term disease-free survival in approximately 50% of patients with chemo-sensitive relapsed lymphoma,[78] this rate seems to decline to approximately 20% for patients with relapsed B-cell NHL after the introduction of Rituximab.[83] While the reasons behind this remain uncertain, it is possible that a selection bias occurs when Rituximab is the chosen treatment in a disproportionate number of cases of resistant disease in the setting of relapse. In this setting, cure-rates appear to be lower, though this population is likely inherently different from patients with lymphomas more responsive to treatment.[2] A more recent review found that the survival rates from relapsed DLCBL with first-line treatment with rituximab at one and five years was 41% and 27%, respectively.[84]

Auto-HSCT may also be employed as consolidative therapy for high-risk lymphomas after their first complete remission (CR1). While not curative, mantle cell lymphoma is often managed with auto-HSCT as it prolongs progression-free survival.[85] The greatest benefit is derived from auto-HSCT after induction with regimens resembling the CHOP protocol (cyclophosphamide, doxorubicin, vincristine, and prednisone), as those induced with more aggressive regimens such as hyperCVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) do not consistently benefit from auto-HSCT in CR1, which may be explained by increased toxicity of the more aggressive regimen.[86] A 2013 study by Delarue et al. found that Rituximab-containing protocols such as R-CHOP and R-DHAP (rituximab, dexamethasone, cytarabine and cisplatin) followed by auto-HSCT showed an improved overall survival of 83 months in patients with mantle cell lymphoma, compared to 51 months for patients on non-rituximab containing protocols.[87] Patients with mantle cell lymphoma should be referred to a

transplant centre early on, as auto-HSCT is most effective when administered early in the disease course.[88]

1.9 Multiple Myeloma

Another common indication, in fact, the most common indication for auto-HSCT in the United States, is MM,[89] a malignant condition characterized by malignant clonal proliferation of plasma cells producing monoclonal proteins and associated with complications such as hypercalcemia, skeletal lesions and bone pain, anemia, renal failure, susceptibility to infection, neurologic symptoms, soft-tissue plasmacytomas and manifestations of hyperviscosity.[9,90] Bone pain, the most common symptom identified among patients with MM, presents in approximately 70% of patients. Should the pain become persistent and localized, it is highly indicative of the presence of a pathologic fracture. Fractures caused by lytic lesions in the bone are a manifestation of the proliferation of tumour cells, osteoclastic activation which breaks down bone tissue, and osteoblastic suppression, preventing the formation of new bone. Lytic lesions may cause the vertebrae to collapse and may result in serious complications, such as spinal cord compression.[90] As a result of bone breakdown, calcium is released into the bloodstream, resulting in further complications secondary to hypercalcemia such as malaise, lethargy, confusion, constipation, and anorexia. Hypercalcemia may also result in end-organ damage, such as renal failure.[91] A plasmacytosis of >10% within the bone marrow or extramedullary plasmacytoma with evidence of monoclonal protein in the serum or urine, as well as evidence of end-organ damage (manifested through hypercalcemia, renal failure, anemia, or bone lesions) or presence of biomarkers of malignancy is diagnostic of MM (eg. increased clonal bone marrow plasma-cell percentage).[92]

Patients with MM are highly susceptible to bacterial infections, most commonly pneumonia caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* or pyelonephritis caused by *Escherichia coli*, or other gram-negative organisms. Infection is so common among patients with MM that approximately 25% will present with recurrent infection, while more than 75% of patients will experience at least one serious infection over the course of their disease. Most patients with MM have hypogammaglobulinemia because of decreased production, reducing the patient's immune response to infection. In addition, the monoclonal protein increases the body's fractional catabolic rate (defined as "the fraction of the administered immunoglobulin in plasma that is catabolized per day,"[93,94] from 2% to 8-16%. The susceptibility to infection is even greater in a subset of patients whose disease creates a population of regulatory cells which are released into circulation and suppress the normal synthesis of antibodies.[90]

The cause of MM remains unknown, though it was observed at rates higher than the general population in those exposed to radiation following the use of nuclear weapons during the Second World War, demonstrating a 20-year latency period before disease manifestations. MM is also observed more commonly among farmers, woodworkers, leatherworkers and those exposed to petroleum products in comparison to the general population, but no mechanism of causation has been established. Specific chromosomal alterations have also been observed in patients with MM, such as hyperdiploidy, translocations such as t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16), 13q14 deletion, 1q amplification or 1p deletion, and 17p13 deletions. There is some evidence that leads us to believe that errors in class switch recombination (a genetic mechanism allowing for heavy chain isotype switching) play a role in the early transformation process

implicated in the pathogenesis of MM, though no single molecular pathway has been determined to be pathogenetic to the disease.[90]

In patients with MM, disease cells bind to bone stromal cells, as well as to the extra-cellular matrix, through cell-surface adhesion molecules. This triggers the growth of the disease cells, as enhances tumour cell survival, drug resistance and promotes migration into the bone marrow milieu. Cytokines produced during this process mediate the growth and survival of MM cells, including anti-apoptotic effects and the development of drug resistance.[90]

1.9.1 Treatment of Multiple Myeloma

Many drugs have demonstrated activity against MM, resulting in a variety of available regimens for the treatment of the disease. Major classes of drugs used in these regimens consist of alkylating agents such as melphalan and cyclophosphamide, corticosteroids such as dexamethasone and prednisone, immunomodulatory drugs such as thalidomide, lenalidomide, and pomalidomide, and proteasome inhibitors such as bortezomib, carfilzomib and ixazomib. In more recent times, we have seen more evidence for the use of monoclonal antibodies such as daratumumab and isatuximab, which target CD38, in the management of MM. Drugs that play a smaller role, but remain approved agents for the treatment of the disease include elotuzumab, a monoclonal antibody targeting the SLAMF7 antigen, panobinostat, a histone deacetylase inhibitor, and Selinexor, an exportin-1 inhibitor. These function poorly as single agents but demonstrate significant therapeutic benefit when combined with other agents. Occasionally, anthracyclines, such as doxorubicin and liposomal doxorubicin are used, but they have minimal single-agent activity in the setting of MM and have largely been replaced by superior options,

though doxorubicin remains a treatment, when combined with other agents, for aggressive or refractory MM.[95]

Eligibility for transplant is determined by the treating physician and is affected by a variety of factors, with age, performance status and co-morbidities being among the most important considerations. Modern treatments often demonstrate a robust response and some patients are able to achieve a minimal residual disease (MRD)-negative state, as defined by specific criteria on imaging, flow cytometry, polymerase chain reaction, or next-generation sequencing, established by the International Myeloma Working Group.[96] MRD negative status is associated with improved progression-free, as well as overall survival in patients with MM.[95]

When considering a management plan for a patient with newly diagnosed MM, the 2 main considerations are whether they are a transplant candidate, and a genetic risk stratification system. Risk stratification is based on cytogenetics; patients with t(4;14), t(14;16) or t(14;20) translocations, as well as 17p deletion, 1q gain or p53 mutation are deemed to be high-risk patients, the remaining mutations being standard-risk.[97] Patients with two high-risk mutations are said to have double-hit MM, while patients with three or more high-risk mutations are said to have triple-hit disease.

In general, auto-HSCT candidates in the standard-risk category are initially treated with three or four cycles of induction chemotherapy, followed by stem cell collection and transplant, followed by maintenance therapy. In select cases, physicians may choose to reserve auto-HSCT in standard-risk patients until relapse. In general, induction therapy with VRd (bortezomib,

lenalidomide, and dexamethasone) is preferred in patients undergoing auto-HSCT as it is well-tolerated and associated with high overall survival and complete response rates. VRd demonstrated improved progression-free survival, as well as overall survival over Rd (lenalidomide and dexamethasone) in a 2017 randomized controlled trial performed by Durie et al.,[98] These findings of improved survival were later confirmed by the authors by longer-term monitoring in a follow-up study.[99] Induction with DRd (daratumumab, lenalidomide, and dexamethasone), is an alternative regimen to VRd for patients with intolerance to the regimen or pre-existing neuropathy, but in the general population, VRd is preferred based on the strength of evidence demonstrating long-term response to therapy.[95,100]

At our centre, a practice consistent with many other Canadian centres, we typically use CyBorD (cyclophosphamide, bortezomib, and dexamethasone) due to budgetary constraints. There is still significant support for the CyBorD protocol as a conditioning regimen for patients with MM undergoing auto-HSCT. A retrospective study by Khan et al. in 2012 found that, when compared to CRD (cyclophosphamide, lenalidomide, and dexamethasone), another option for induction, CyBorD demonstrated superior response rates with less toxicity.[101]

For high-risk patients who are eligible for transplant, daratumumab is generally recommended in conjunction with standard VRd therapy, as the GRIFFIN trial demonstrated improved response to therapy such patients over VRd therapy.[102] VRd therapy recently became available for patients in Canada; however, daratumumab has yet to receive funding in our country as first-line therapy, and is only available to those who have failed other therapies.[103]. Another regimen, Dara-VTd (daratumumab, bortezomib, thalidomide, and dexamethasone) may be considered in this group of

high-risk patients, as it was associated with improved overall survival when compared to VTd (Bortezomib, Thalidomide, and Dexamethasone), the standard therapy used for transplant-eligible MM patients in Europe.[104]

For patients with MM who are ineligible for transplant, induction therapy generally consists of VRd or DRd, as melphalan-based regimens have gone out of favour due to the potential for stem cell damage, secondary myelodysplasia and acute leukemia. VRd is generally considered the preferred regimen, and has demonstrated improved overall survival over Rd alone,[98] and at 8-12 cycles, is a much shorter course and less burdensome treatment when compared to DRd, which requires continued treatment with daratumumab, lenalidomide and dexamethasone until disease preogression.[95] Lower doses of lenalidomide and dexamethasone may be used in frail elderly patients, at the discretion of the treating physician, as well as other substitutions based on co-morbidities and drug availabilities. DRd has also demonstrated superiority over Rd in terms of progression-free survival,[105] though this regimen must be continued until disease progression, resulting in a long-term commitment to therapy, which can be inconvenient and onerous for the patient, as well expensive to the health-care system, and is therefore not yet funded in Canada.[100,103,106]

1.9.2 Evidence for Autologous Stem Cell Transplant in Multiple Myeloma

Although auto-HSCT is not a curative treatment in the setting of MM, it has been shown to improve the median overall survival by approximately 12 months,[107–114] with a relatively low treatment-related mortality rate at 1-2%. Patients undergo a conditioning regimen prior to transplant.[95]

A 2014 review article by Gertz and Dingli[89] outlined the use of auto-HSCT in MM. They discussed 2 major studies which showed a 12-month improvement in overall survival in patients receiving auto-HSCT over those that had not, though these studies do predate novel agents.[107,108] While auto-HSCT is not a curative therapy in the setting of MM, many randomized controlled trials demonstrate improvement of event-free survival with auto-HSCT.[107,108,115] A 2013 randomized controlled trial by Palumbo et al. involving 389 patients found that patients who had received lenalidomide induction therapy and auto-HSCT in the setting of MM had a significant improvement in progression-free survival at three years over patients who received a consolidation regimen of cyclophosphamide, dexamethasone and lenalidomide after their lenalidomide induction therapy.[116]

Data from The Center for the International Blood and Marrow Transplant Registry showed that there has been an overall decrease in mortality post auto-HSCT over time; survival at 60 months was measured to be 47% when analyzing the data from 1995 to 1999, 55% from 2000 to 2004 and 57% from 2005 to 2010. They attribute this partly to auto-HSCT practices themselves and partly due to the utilization of novel agents such as bortezomib and immunomodulatory derivatives such as thalidomide and lenalidomide.[106,117]

1.10 Conditioning Regimens

A conditioning regimen is a preparatory regimen prior to a HSCT which comprises a treatment of chemotherapy and/or radiation therapy which eradicates the patient's native bone marrow including their own hematopoietic and immune cells as well as any malignant cells.[4] This

treatment aims to both eliminate any malignant cells from the body and provide sufficient immunosuppression to allow for donor engraftment,[9]though is not considered a curative treatment as progression and relapse of disease remains common, even when complete remission is attained.[118]

There are 2 types of conditioning regimens: myeloablative and non-myeloablative. The goal of a myeloablative conditioning regimen is to completely and irreversibly eliminate the patient's own hematopoietic function within their own bone marrow using a combination of high-dose chemotherapy with TBI. TBI may be administered in a single dose or may be fractioned and delivered over the course of several days. In terms of chemotherapy, several protocols exist which use agents such as cyclophosphamide, busulfan, melphalan, cytosine arabinoside, etoposide or nitrosoureas. Patients must then wait a minimum of 36 hours after the last dose of chemotherapy is administered before receiving their stem cell transplant.[4]

Non-myeloablative regimens were developed in response to the significant morbidity and mortality associated with myeloablative regimens because of complications such as mucositis, sometimes requiring parenteral nutrition. Non-myeloblative conditioning avoids the complete destruction of the patient's own bone marrow. Lower-dose radiation may be used in conjunction with agents such as fludarabine, antilymphocyte globulin or other antibodies acting on T-cells. Agents used in myeloablative regimens such as cyclophosphamide or busulfan may also be used in non-myeloablative regimens, but they are administered at much lower doses. The goal of these regimens is to induce enough immunosuppression in order to allow for donor cell engraftment, but minimize complications inherent to myeloablative regimens.[4]

The specific conditioning regimen used will depend on the type of transplant (i.e. typically more intense treatments are required for allo-HSCT with unrelated and mismatched transplants with milder treatments reserved for auto-HSCT or sibling-matched allo-HSCT). Diagnosis also affects choice of conditioning regimen, especially in auto-HSCT where anti-disease specific chemotherapeutic agents such a melphalan in the setting of MM or carmustine or cyclophosphamide in the setting of lymphoma are used. While these are the main considerations when choosing a conditioning regimen, age, recipient co-morbidities and disease-specific toxicities are also taken into consideration.[9]

The most common indications for auto-HSCT are MM and lymphoma. One of the most popular conditioning protocols in preparation for auto-HSCT is the BEAM protocol which consists of carmustine (1,3-bis (2-chloroethyl)-1-nitrosurea), etoposide, cytarabine and melphalan. This is most commonly used for patients with lymphoma receiving auto-HSCT.[119,120] This regimen is used at our centre. There are significant toxicities associated with these medications; carmustine carries risk of pulmonary toxicity, etoposide, hepatic toxicity and cytarabine, cardiac toxicity. These agents have superior tumour-killing abilities at higher doses, but toxicities must be taken into account, therefore, they are administered at levels lower than the maximally tolerated doses in combination to minimize toxicity, but maximize their ability to kill tumour cells and overcome drug resistance.[2] For the majority of patients undergoing auto-HSCT in the setting of lymphoma; however, the conditioning regimen is altered slightly for the few patients found to have central nervous system disease. These patients are treated with busulfan, melphalan and thiotepa, with or without the addition of rituximab. A 2008 study by Zaucha et al. compared BEAM conditioning

to BuMelTT (busulfan, melphalan, and thiotepa) and found similar death, relapse and treatment failure rates in both groups.[121]

The preferred conditioning regimen, as well as the conditioning regimen used at our centre for MM, is high-dose melphalan.[9] There is some evidence that other regimens may take precedence over high-dose melphalan in the future; a 2019 trial performed by Bashir et al. showed a median survival of 64.7 months using busulfan and melphalan, compared to a 43.5 month median progression-free survival with melphalan alone,[122] with superior outcomes confirmed in 2020 by Saini et al.[123] A 2019 trial by Gomez-Arteaga proposed bendamustine and melphalan as another alternative to melphalan conditioning prior to auto-HSCT in the setting of MM. Though not a randomized-controlled trial, the study generated promising results, demonstrating a median progression-free survival of 48 months in newly diagnosed MM. Results were even more promising in patient with relapsed and refractory myeloma, as subgroup analysis revealed a median survival of 45 months.[124]

1.11 Selection Criteria for Transplant Candidates

Selecting appropriate transplant candidates involves an in-depth holistic assessment of the patient considering such factors as diagnosis, response to previous treatments, as well as psychosocial factors. Patients are typically referred for consideration of transplant by their hematologist, though the evaluation for and counselling given prior to a transplant is best approached from a multi-disciplinary perspective, involving physicians, nurses, social workers, patients, families and caregivers, among others. Many factors will weigh into the decision to

proceed with transplant or not, including disease status at the time of transplantation, age, and medical co-morbidities.[2]

1.11.1 Imaging

One of the most important predictors of long-term outcomes including disease-free survival is disease status at the time of transplantation. For patients with DLCBL, for example, FDG-PET imaging prior to transplantation acts as a predictor for disease-free progression in those receiving auto-HSCT.[80,125–127] A 2012 study by Moskowitz et al. found that patients with a negative FDG-PET prior to auto-HSCT for relapsed or refractory HL had >80% rate of event-free survival in contrast to their counterparts with positive scans, who had only a 28.6% rate of event-free survival.[80] Other disease-specific prognostic factors include cytogenic and molecular abnormalities, immune-phenotype, as well as any evidence of extra-nodal or extra-medullary disease.[2]

1.11.2 Age

MM is a disease that typically affects the elderly, with only 35% of diagnosed cases being in patients younger than 65 years.[106] Age is relevant to the treatment of MM. Age has previously been reported as an independent prognostic factor for outcomes in patients with MM,[128] but age is also a factor when trying to anticipate tolerance of high-dose therapy and auto-HSCT.[106] Since the success of an auto-HSCT is contingent on the use of a high-dose conditioning regimen to eradicate tumour cells, the conditioning regimen cannot be reduced effectively for older or more frail patients.[2] In recent years, however, we have moved away from age being an independent criterion for selection for transplant eligibility in favour of the consideration of frailty instead. In

a study published in 2022, Barth et al. found that age had no significant impact on transplant outcomes, the outcomes in question being greater mean transplant hospital day, greater total hospital days or greater risk of ICU (intensive care unit) admission.[129]

1.11.3 Comorbidities

Medical co-morbidities at the time of transplant are also an important factor when considering hematopoietic stem cell transplant for a particular patient. Patients must undergo routine screening for major organ systems such as cardiac and pulmonary function since any deficiencies may play a critical role when determining whether a patient will be able to tolerate therapy. Renal and hepatic screening, as well as infectious disease screening for such viruses as cytomegalovirus (CMV), hepatitis B, hepatitis C, herpes simplex virus (HSV), and human immunodeficiency virus (HIV) should also be performed in all patients. Nutritional status at either extreme, such as cachexia or obesity can also negatively impact transplant-related mortality.[2,130,131] Several scoring systems are used to facilitate the quantification of co-morbidities in order to predict success with transplant. The European Group for Blood & Marrow Transplantation (EBMT) Risk Score,[8] the Pretransplant Assessment of Mortality (PAM) score,[132] and the HCT-specific Comorbidity Index (HCT-CI)[133,134] are three of the most common pre-transplant co-morbidity assessment tools used today.[2] The HCT-CI is a transplant-specific modification of the Charlson Comorbidity Index(CCI). The CCI is a tool used at our centre.[135] The tool has been validated in allo-HSCT, however, a recent study demonstrated that the HCT-CI, age-adjusted or not, did not predict transplant outcomes such as days in hospital attributable to transplant, total days in hospital or ICU admission.[129] In a 2020 study of 131 patients ages 65-75 being considered for auto-HSCT, Belotti et al.also found that while the HCT-CI and CCI did

not predict outcomes in these patients, another frailty index, the International Myeloma Working Group (IMWG) Frailty Score did help to identify patients aged 70-75 who were not appropriate auto-HSCT candidates.[136]

1.11.4 Functional Status

Performance status is another important prognostic factor to consider in the evaluation of transplant suitability. The Karnofsky Performance Score (KPS)[137,138] and the Eastern Cooperative Oncology Group (ECOG) Performance Status[139] are frequently employed as measures of functional status. The KPS was first published in 1949 and consists of 11 levels ranging from zero to 100 in increments of 10 with 100 representing a normal functional status of a healthy person with no complaints or evidence of disease and zero representing a patient who is dead. The ECOG score was first published in 1982. It ranges from zero to five with a score of zero corresponding to a KPS of 100, where the patient is fully active and able to carry on all pre-disease activity with no restrictions and an ECOG score of five corresponding to a KPS of zero, where the patient is dead.[140] In 2012, The American Society of Clinical Oncology (ASCO) assembled a panel of experts to discuss the use of chemotherapy among oncology patients for whom there was no evidence of clinical benefit. They established that this was a wasteful and unnecessary practice that was rampant within oncology practice and recommended that performance status be used as an indicator to gauge whether or not chemotherapy would be of benefit to a particular patient.[141] The ASCO guidelines recommend against the use of chemotherapy in patients who have not benefitted from previous treatment and who have an ECOG score of at least three, meaning that they are capable of only limited self-care and are

confined to their bed or chair for >50% of waking hours.[140,142] These performance scales are also routinely used in the setting of hematologic malignancies.[143]

1.12 Tumour Contamination in Autologous Stem Cell Transplant

Concerns always remain surrounding the potential for residual tumour cells acting as a source of contamination to the PBPC product which has the potential to contribute to a relapse when re-infused into the patient, though it is very difficult to distinguish the relative contributions of graft contamination and residual disease in the patient to failure of treatment or relapse. Researchers have attempted to mark the cells within the PBPC product at the time of harvest to be able to assay this marker gene in malignant cells should the patient relapse. The success of these attempts is unclear, and different studies have led to different conclusions. Alici et al. were not able to identify any marked myeloma cells in six patients while in remission nor during relapse, which led them to conclude that graft contamination is unlikely to contribute to relapse.[144] Brenner et al., however, studied two children with acute myeloid leukemia who received auto-HSCT and found that both patients relapsed, and both had marked cells present at the time of relapse, concluding that graft contamination may indeed play a role in disease relapse.[145]

Researchers have looked at several disease-specific means of lowering this risk including the administration of rituximab prior to collection, *ex vivo* positive selection of CD34+ cells, chemotherapy-based purging using cyclophosphamide derivatives and purging through oncolytic viruses. Kasamon et al. found that the incorporation of hydroperoxycyclophosphamide (4-HC) purging into initial or salvage therapy produced extended periods of remission in some patients with indolent lymphomas.[146] Bartee et al. studied the use of an oncolytic poxvirus as a means to

eliminate myeloma cells by inducing apoptosis in these cells, selectively, while sparing the normal hematopoietic stem cells from cell death, as the virus is unable to bind with or infect CD34+ cells. Animal studies showed that use of this virus was a safe and effective means to selectively eliminate malignant cells.[147] Yahng et al. retrospectively analyzed the disease course of 56 patients with NHL, 24 of whom were infused with a purged cellular product, and 32 who received the regular cell product. The purging was completed by positive selection of CD34+ cells using a magnetic-activated cell sorting machine. They demonstrated a significant improved 3-year overall survival as well as progression-free survival when using the purged cellular product.[148] Gribben et al. used PCR to detect any residual lymphoma cells before and after the immunologic purging procedure. All 114 patients had detectable lymphoma cells within their bone marrow prior to the procedure while after purging, 57 patients did not have any detectable lymphoma cells in their marrow. This disease-free group of patients did benefit from a significant improvement in disease-free survival. The authors found that the inability to purge lymphoma cells within the marrow was the single most important factor predicting disease relapse.[149] Finally, Pettengell et al. found the rituximab administered prior to collection failed to improve progression-free or overall survival, but when administered as a maintenance therapy after auto-HSCT, rituximab demonstrated a progression-free survival benefit in patients with relapsed follicular lymphoma, though this did not translate into improved overall survival.[150] Overall, some evidence does exist to suggest a benefit to purging, though few randomized controlled trials exist to support this practice.[2] One randomized controlled trial did investigate the use of rituximab as an *in vivo* purging method for patients with NHL receiving auto-HSCT in comparison to *ex vivo* purging strategies, and showed that it is likely safer than *ex vivo* methods as it did not impair CD34+ cell mobilization and demonstrated faster myeloid cell recovery.[151]

There is also a small potential for harm with some purging techniques; the process depletes the cell product of mature T-cells which can increase the risk of infections, especially CMV and the reactivation of other viral diseases, post-transplant.[152,153] Due to the lack of conclusive evidence of its benefit, purging strategies are generally not applied to auto-HSCT products at this time.[2]

1.13 Cryopreservation

Once the patient's stem cells have been collected, the cells are cryopreserved to allow the patient to undergo their conditioning regimen in preparation for the infusion of the transplant a few weeks later. While collected cells can be stored for up to 72 hours post collection at temperatures ranging between 2°C and 6°C, it is recommended that cells be cryopreserved within 48 hours of collection to maintain cell viability. Guidelines also recommend that the concentration of nucleated cells in the collection should not exceed 4 x 10^8/mL, so that the product may be diluted with autologous plasma or with commercial formulations. Dimethyl sulfoxide (DMSO) is added to the product as a cryoprotectant. Cells should be stored in vapour-phase nitrogen at a temperature no colder than -140°C, though the cells must arrive at that temperature at a controlled rate of 1-2°C per minute.[9] Cryopreserved stem cells are thawed at the site of transplantation, as they should ideally be reinfused into the patient within 20 minutes of thawing.[9]

While controlled-rate freezing (CRF) is the standard of care at most facilities, it is certainly more expensive and cumbersome than another cryopreservation technique – uncontrolled-rate freezing (UCF). A 2018 study recognized that several studies had already taken place casting doubt on whether CRF was necessary to preserve cellular integrity or whether so-called "dump freezing"

would be a less expensive and less time-consuming alternative for cryopreservation in areas where resources were scarce. UCF involves creating a cryoprotectant solution (using 5% dimethyl sulfoxide, 5% albumin and 2% hydroxyethyl starch) and storing the cell product at -80ºC. The authors found, through their own trial, that UCF could indeed be a practical solution for cryopreservation when a lower-cost solution is necessary; cell viability is widely variable even across studies using CRF, and results of this study showed the viability of cells cryopreserved using UCF also fall within this spectrum of viability.[154]

1.14 Engraftment

The goal of any stem cell transplant is to eliminate the patient's own diseased bone marrow and replace it with donor marrow which will, hopefully, engraft into the patient's bone marrow and resume hematopoietic and immunologic functions. Inevitably, a period of profound pancytopenia is the initial result of a bone marrow transplant prior to engraftment. This period typically lasts one to three weeks, though this period may be prolonged. Monocytes and neutrophils usually start showing engraftment with rising blood counts earlier than other types of cells, with platelet count following. A reticulocytosis can subsequently be observed. The earliest lymphocytes to appear are typically the natural killer (NK) cells. The engraftment period using the bone marrow as a collection source is typically longer than when stem cells are harvested from the peripheral blood. Due to the high risk of opportunistic infection while the patient is neutropenic, G-CSF can be used to help the neutrophils to proliferate while the patient is neutropenic.[4]

Myeloid engraftment is most commonly defined as the first of three consecutive days on which the absolute neutrophil count measures $\geq 0.5 \times 10^9$ cells/L. It typically occurs within 21 days of

stem cell infusion regardless of the type of transplant or source of hematopoietic stem cells. The definition of platelet engraftment is more nebulous; it may be defined as the first of seven consecutive days with a platelet count ≥20, 50, or 100 x 10^9 platelets/L, maintained without the support of platelet transfusion. Platelet recovery may occur significantly after myeloid recovery, especially following grafts with low CD34+ cell doses. We generally define erythrocyte engraftment as a hemoglobin of >80 g/L without the support of transfusion, though the number of days this must be sustained is ill-defined.[2]

Graft failure occurs when donor hematopoietic cells do not engraft after stem cell transplant. It may be early (or primary) failure, or secondary (or late) failure depending on temporal factors. Graft failure of any type carries significant morbidity and mortality with a high risk of death secondary to hemorrhage, infection or relapsed malignancy.[2] The risk of graft failure in the setting of auto-HSCT is significantly lower than in allo-HSCT, but may still occur. Overall, <5% of auto-HSCT results in mortality, and of those only a small subset are related to graft failure. We can estimate graft failure in auto-HSCT by quantifying how many patients require a "back-up" autograft product due to failure of the first attempt. A 2002 study by Pottinger et al. found that of the 300 patients who received an auto-HSCT in their study, only 14 (4.7%) required a rescue cellular product after either primary or secondary graft failure.[155] Significant mortality may arise post auto-HSCT from prolonged cytopenias resulting from graft failure. Patients undergoing auto-HSCT have already received high-dose chemotherapy and are completely dependent on hematopoietic reconstitution from their autograft.[2]

1.15 Complications after Autologous Stem Cell Transplant

1.15. 1 Mucositis

Most patients who have received high-dose chemotherapy will exhibit some degree of mucositis. Management is mainly supportive including saline and antimicrobial rinses, cryotherapy and medications for pain control, and escalated to parenteral nutrition, when required. Patients typically see improvement in their condition around the time of engraftment, approximately 10-21 days post-transplant. The mucositis tends to be more severe with the use of fully ablative regimens, TBI-based conditioning, and the use of post-transplant methotrexate for prevention of graft-versus-host disease (GVHD),[156] and can occasionally manifest as soft tissue edema, upper airway obstruction and aspiration pneumonia. Mucositis also compromises the mucosal lining, which can potentially result in bacterial translocation from the gastrointestinal tract and subsequent bacteremia.[2]

1.15.2 Sinusoidal Obstructive Syndrome

Sinusoidal Obstructive Syndrome (SOS), formerly referred to as veno-occlusive disease, is characterized by tender hepatomegaly, fluid retention and weight gain, as well as hyperbilirubinemia. The sinusoidal epithelium of the liver is damaged through cytoreductive therapy.[157] Cytarabine is often implicated in the development of SOS, though it is difficult to determine which patients will become symptomatic as the metabolism of cytarabine varies a great deal from patient to patient,[158,159] and this toxicity may be further exacerbated by co-administration with busulfan[160] or pre-existing hepatic fibrosis.[161] SOS is often mild and self-limited, though in moderate disease, SOS that does not resolve spontaneously before day 100 post HSCT, diuretics and pain medication are required. Occasionally SOS may be severe and can

cause multi-organ failure and death with a mortality rate of >80%.[2,162,163] Severe SOS is difficult to treat with few effective therapies available. Defibrotide, an antithrombotic and fibrinolytic is FDA-approved for SOS based on a small trial showing short-term improvement in mortality, but there is no evidence to suggest its effectiveness in improving longer-term survival.[164–166]

1.15.3 Pulmonary Injury

Approximately 10-15% of patients will develop idiopathic pneumonia syndrome (IPS) following HSCT.[167] IPS is an acute following by a chronic inflammatory state within the lungs that lead to fibrosis.[168] Though containing the term *pneumonia*, IPS is not a result of infection. Patients are at higher risk for this condition after undergoing high-dose conditioning or TBI, having GVHD, or having a pre-disposing lung condition such as prior smoking history, thoracic or mediastinal radiation or abnormal gas exchange seen on pulmonary function testing.[169] Occasionally, diffuse alveolar hemorrhage (DAH) may develop from IPS. Patients with DAH present with progressive dyspnea and hypoxemia and will demonstrate progressively bloodier findings on bronchoalveolar lavage (BAL). Mortality is high, often more than 75% of patients. Another subset of IPS patients may develop peri-engraftment respiratory distress without hemorrhage. Patients with IPS following auto-HSCT often respond to glucocorticoid and broad-spectrum antibiotic treatment, when administered promptly, in contrast to allo-HSCT patients whose response rates are generally poorer.[2]

Afessa et al. performed a retrospective chart review of 1243 patients who had received auto-HSCT and found that 27.6% of these patients developed some type of pulmonary complication. Within this group, 50.4% of cases demonstrated an infectious etiology, 40.0% non-infectious and

the remaining were a mixed picture involving both infectious and non-infectious etiologies. The main non-infectious etiologies were acute pulmonary edema, followed by DAH, then peri-engraftment respiratory distress syndrome and IPS. The authors identified lung diffusion capacity for carbon monoxide and indication for transplant as independent risk factors for pulmonary complications post auto-HSCT. Risk factors for mortality from pulmonary complication post auto-HSCT include male sex, history of pulmonary disease prior to transplant, advanced disease status, lower forced vital capacity (FVC) and poorer Karnofsky performance status.[170]

1.15.4 Infection

Fever during the neutropenic period immediately following HSCT occurs in almost all patients. Neutropenic patients are at particular risk for bacteremia and there is a particularly high mortality rate associated with Gram-negative bacteremia should there be any delay in the initiation of antibiotic treatment. Neutrophils are responsible for the clinical signs that are indicative of localized bacterial infection such as abscess formation, lung infiltration, and pyuria and in their absence, fever may be the only sign of infection, which is unfortunately very non-specific. Fever may occur due to a wide variety of etiologies such as bacterial, viral or fungal infection, or even non-infectious causes such as drug or transfusion reaction, engraftment syndrome, GVHD or rejection. This poses a challenge to clinicians to correctly identify the cause of fever with very few clinical signs or symptoms attached. It is for this reason that febrile neutropenia is considered a serious occurrence post-transplant and is addressed in a prompt, thorough and systematic fashion by clinicians. At least 2 sets of blood cultures are collected immediately, along with a thorough patient history and physical examination to identify any potential focal

sources of infection. If clinically indicated, physicians may choose to order a sputum culture, nasopharyngeal swab for respiratory viruses, among others. Following this testing phase, patients are treated with broad-spectrum antibiotics with the potential of anti-viral or anti-fungal therapy if there is a high clinical suspicion.[9]

1.15.4.1 Bacterial Infection

Patients are at particular risk for bacterial infection during the period of neutropenia prior to neutrophil engraftment and bacteremia is thought to be the cause of fever in approximately 25% of all neutropenic patients.[171,172] Patients are at additional risk of bacterial infection due to epithelial injury secondary to chemotherapy, subsequent bacterial translocation and the necessity for various types of indwelling catheters. Handwashing, gowning, gloving and masking are often employed as a means to reduce exposure to patients.[2] The management of febrile neutropenia is further complicated by emergency multidrug-resistant (MDR) organisms such as *Enterobacteriaceae* which are now showing increasing resistance patterns to typical antibiotic regimens such as third-generation cephalosporins, carbapenems, and piperacillin-tazobactam, with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* also demonstrating resistance to carbapenems.[9]

Patients remain at high risk of infection during the three to 12 months following a transplant as there are few CD4+ helper cells present in the marrow at this time.[4] Patients must be diligently monitored for any signs of infection post HSCT, and are generally also treated with a variety of preventative measures such as prophylactic antimicrobials therapies. Immune recovery following auto-HSCT is relatively quick when compared to those receiving allo-HSCT.[2,173] Over time, the

marrow recovers, and cellularity rises to within normal limits, though the marrow does not completely recover for a period of one to two years.

Rahman et al. performed a retrospective chart review of 413 patients who had received an auto-HSCT for treatment of their MM. They identified infection as the most common cause of in-hospital mortality in this group of patients. In this study, 21% of patients in the study experienced at least one infection in the first 100 days after their transplant with the most common etiology being bacterial (75%) and 67% of these infections were attributed to Gram-positive bacteria, with the remaining being associated with Gram-negative bacteria. The most common organisms isolated were *Clostridioides difficile*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Patients with infection spent more days in hospital following their initial transplant and were re-admitted to hospital more frequently after discharge.[174]

Neutropenic enterocolitis is a rare and potentially fatal complication following high-dose chemotherapy seen post auto-HSCT. Disease is typically incited by infection with gram-negative bacteria. In a study of 297 patients who received auto-HSCT for their hematologic malignancy, 262 (88%) patients developed neutropenic infections post-transplant with neutropenic enterocolitis representing 12% of these infections. Nine patients developed ileus or septic shock and the disease proved fatal in three cases despite treatment with carbapenem antibiotics and bowel rest. Neutropenic enterocolitis was found in disproportionately high numbers in patients having been conditioned using the BEAM regimen.[175]

1.15.4.2 Viral Infection

Varicella zoster virus (VZV) infections are commonly seen in patients after auto-HSCT, with some guidelines suggesting prophylaxis against VZV for one year post-transplant. Rogers et al. investigated the rate of reactivation of the virus in 56 patients post auto-HSCT and found that 16% of patients experienced reactivation, which had a median onset of 4.5 months post-transplant (two to 10 month range). Complications of the virus include post-herpetic neuralgia, bacterial coinfection, neuropathic pain, scarring, hyperpigmentation, weakness, keratitis, vision loss and neurological dysfunction.[176]

CMV may also appear at around two to three weeks post HSCT.[177] Infection may occur *de novo* through donor graft, in the setting of allo-HSCT, or blood transfusion, or it may be a reactivation of the latent virus. CMV is a relatively rare infection after auto-HSCT, though it may occur in the setting of CD34+ selection or T-cell depletion.[152] Following auto-HSCT, there is a transient early reconstitution of immunologic function that is thought to be a result of the proliferation of mature T cells that managed to survive the conditioning regimen, or were perhaps transferred to the patient via the autograft. CD34+ selection of stem cell grafts reduces the number of available T-cells, which could potentially delay hematopoietic reconstitution and result in increased risk of infection.[178] A higher incidence of CMV infection has been reported among patients who have received CD34-selected autografts. Crippa et al. expanded on this theory and found that in addition to CMV infection, VZV and parainfluenza three virus infections were also more prevalent in this population.[153]

A 2019 study of 196 patients by Balsat et al. looked at human herpesvirus 6 (HHV-6) in patients who had received an auto-HSCT for their hematologic malignancy and found infection in 11.2% of patients. HHV-6 infection resulted in a significantly longer period of hospitalization (30.5 days for those with infection, 22 days for those without) than their counterparts who were not infected. Infected patients also required transfusions later on in their post-transplant course than non-infected patients.[179] Colombier et al. produced similar results in a review of 316 patients treated with auto-HSCT for lymphoma. Reactivation of HHV-6 was reported in 8.5% of patients who developed symptoms such as fever, diarrhea, rash and pneumonia.[180]

In 2020, Kim et al. published a case report investigating disseminated adenovirus infection in patients after auto-HSCT. They acknowledged that though adenovirus infection after auto-HSCT is rare, the consequences can be devastating, with a mortality rate of up to 26%.[181]

1.15.4.3 Fungal Infection

Fungal infections are a potentially life-threatening complication following any type of HSCT, though they are more common in allo-HSCT because of the immunosuppressive medications required after allo-HSCT. The incidence of fungal infection is highly dependent on the centre, geography location, local hazards, and the use of prophylactic regimens post-transplant. The most common pathogens seen are *Candida* and *aspergillus*.[2] Patients treated with prophylactic fluconazole demonstrate a lower incidence of infections caused by *Candida albicans*, and a 2007 systematic review and meta-analysis by Robenshtok et al. suggests a decrease in 100-day all-cause mortality in patients who received allo-HSCT in those patients who received fungal prophylaxis.[182]

Fungal infection is less common following auto-HSCT. The Infectious Diseases Working Party (AGIHO) within the German Society for Hematology and Medical Oncology (DGHO) published a set of guidelines based on expert consensus to address the need for antimicrobial prophylaxis in patients who had received auto-HSCT. They suggested that there was insufficient evidence to support the routine use of prophylactic antifungals in patients who had received auto-HSCT, though it could be considered on a case-by-case basis. The guidelines do moderately support the use of trimethoprim/sulfamethoxazole to prevent *Pneumocystis jirovecii* pneumonia, though they note that the evidence on which this recommendation is based is of low quality.

1.15.2 Thrombocytopenia

A 2020 study by Wada et al. found that patients with central nervous system (CNS) lymphoma treated with busulfan and thiotepa were at risk of secondary failure of platelet recovery (SFPR), with three of 24 patients (12.5%) of patients in the study developing this complication. Of the 50 patients who received the MEAM protocol (ranimustine, etoposide, cytarabine, and melphalan) or high-dose melphalan conditioning, there were no instances of SFPR, though one of the two patients conditioned with busulfan and melphalan did develop SFPR, suggesting an association between busulfan and SFPR.[183]

1.16 CD34+ Cell Count for Quality Assessment

As discussed earlier in this chapter, we use CD34+ cell count as a marker of adequacy for transplant. Hematopoietic stem cells are morphologically indistinguishable from lymphocytes, therefore, alternate means of testing must be used to identify which cells are stem cells and

which cells are not. As a means of distinguishing these cells, we identify the surface marker CD34, which is found on the surface of stem cells.[5] The exact phenotype of multipotent stem cells has yet to be discovered, but on immunological testing, these cells are positive for CD34, negative for CD38 and negative for lineage markers, so we generally use CD34 positivity as a surrogate marker for the identification of stem cells.[4]

CD34+ cell count within peripheral blood collections is used as a measure of graft quality for auto-HSCT.[184] A study by Weaver et al. suggests that the kinetics of neutrophil and platelet engraftment post-transplant are determined by the CD34+ cell dose per kilogram of body weight of the recipient.[185] According to the EBMT Handbook, the principal means of determining the adequacy of a transplant is through a quantification of the CD34+ cells present in the HSCT product.[9] The generally accepted threshold for transplantation is >2.0 x 10^6 CD34+ cells/kg of body weight, though most transplant centres prefer a dose of 2.5-6.0 x 10^6 CD34+ cells/kg.[4,186–190] It is possible for engraftment to occur at lower doses, though studies have shown that platelet engraftment is more profoundly affected by CD34+ cell count, and Duong et al. suggest that a CD34+ cell dose >4 x 10^6 CD34+ cells/kg may be the optimal threshold, as it may result in faster engraftment, reduced rates of infection and non-relapse-related mortality.[186] A 2011 study by Stiff et al. found that while short-term engraftment of neutrophils and platelets was relatively unaffected by CD34+ cell dose, they found that there was a significant association between cell dose and a platelet count >150 x 10^9/L at 100 days, 6 months and 1 year post auto-HSCT.[191]

In 2015, Sauter et al. reviewed the available data on the topic and concluded that while there are data to suggest that CD34+ cell count is associated with hematopoietic recovery, few studies

considered the clinical endpoints of significance. Our study will look more closely at these clinically significant endpoints.

1.17 Study Rationale

Currently at our facility, the adequacy of the collection is assessed through measurement of the CD34+ cell count within the collection, with a cell count of >2.0 x10^6 CD34+ cells/kg of the patient's body weight determined to be an acceptable threshold for transplant. Should the initial collection be deemed inadequate, subsequent collections will ensue until an adequate number of CD34+ cells are harvested for transplantation.

Because of the considerable time and manipulation of the cells between pre-freeze CD34+ cell count and post-thaw viable CD34+ cell count measurement, we wonder if our dependence on a pre-freeze measurement is an accurate representation of CD34+ cells within the transplanted product that the patient receives. The cell count used to determine adequacy of transplant is the pre-freeze CD34+ cell count, though cells must then undergo a lengthy freezing and thawing process prior to administration to the patient. Furthermore, there remains some debate as to whether actual CD34+ counts actually correlate with superior clinical outcomes at all. This study aims to determine whether the post-thaw viable CD34+ count correlates with pre-freeze count and the day of engraftment of platelets and neutrophils.

Considering that our policies dictate that we must obtain these post-thaw cell counts, even though they currently do not affect our clinical practice, we decided to embark on this study to determine if the number of viable CD34+ cells within transplanted products is a helpful measure

at all. If it is indeed a decent predictor of patient outcomes, then we endeavour to investigate how we can actually use these results in our clinical practice. If this is not a helpful measure, as we hypothesize, we aim to share our findings with our institution, providing the institution with evidence that this costly and time-consuming process offers us no clinical benefit and we will have to address alternative quality indicators.

This study aims to determine whether post-thaw CD34+ cell count in auto-HSCT is correlated with clinical outcomes, mainly time to white blood cell and platelet engraftment. Should viable CD34+ cell count not be found to be a helpful clinical indicator, this will act as an impetus for further investigation by the institution and possible abandonment of the current expensive and time-consuming quality indicator for a more clinically relevant one.

Chapter 2: Methods

2.1 Research Objectives

The primary purpose of this study is to determine whether the viable CD34+ cell dose measured within an auto-HSCT post-thaw is correlated with earlier engraftment. Engraftment is defined as the reconstruction of recipient hematopoiesis with white blood cells and platelets from a donor, in the case of auto-HSCT, the patient acts as their own donor. The length of time to engraftment of both white blood cells and platelets serves as a marker of product integrity as, by successfully engrafting, the ability of these cells to perform their intended hematopoietic function is demonstrated.

Our primary outcome will be the date of engraftment for each cell line (neutrophils and platelets). The date of absolute neutrophil count (ANC) recovery is defined as the date of the first of 3 consecutive daily laboratory values where the ANC $\geq 0.5 \times 10^9$/L. Initial platelet recovery data is defined as the first of three consecutive daily laboratory values of platelet count $\geq 20 \times 10^9$/L without platelet transfusion for at least seven days prior to this date. These markers will act as our primary outcomes.

The secondary outcomes of the study will investigate whether post-thaw viable CD34+ cell and pre-freeze CD34+ cell count correlation and whether post-thaw viable CD34+ cell count is associated with ICU admission, rate of infection, and rate of platelet transfusion.

2.2 Methodology

2.2.1 Setting and Study Design

The Health Sciences Centre is a tertiary care facility in St. John's, NL, Canada. It hosts a hematology department which is capable of performing auto-HSCT. To determine whether our current quality assurance measures affect patient outcomes, we completed a retrospective chart review of all patients who received an auto-HSCT as a means of treatment for their malignancy at the Health Sciences Centre in St. John's, NL, between January 2014 and August 2019. We chose January 2014 as our start date because our centre underwent two major changes at that time; cell products, which had previously been collected and delivered as full-volume products were now reduced in volume and the freezing process changed from "controlled" to "dump" freezing, which will be described in greater detail below.

2.2.2 Study Population

Our study population includes adults ≥18 years old who have received an auto-HSCT at the Health Sciences Centre in St. John's, NL between January 2014 and August 2019. These patients principally have diagnoses of MM or lymphoma, though this also occasionally includes patients with a germ cell tumour. Patients were excluded if either pre-freeze or post-thaw data was not available.

2.2.3 Data Collection and Storage

We collected the following data points: Pre-freeze CD34+ cell count, post-thaw viable CD34+ cell count, day to engraftment of neutrophils, day to engraftment of platelets, infection rate (defined as presence of a positive culture, or febrile illness), admission to the ICU and the

necessity for platelet transfusion. We also collected descriptive statistics for each patient including biological sex, age, co-morbidities (measured with the CCI), performance status (measured with the ECOG Performance Status), indication for transplant (ie. hematologic diagnosis) and conditioning regimen. We subsequently amended our data collection form to include KPS as we found that we were missing data when collecting ECOG Performance Status for each patient. Data was collected from patient charts kept by the Hematology Department and supplemental data was obtained using Meditech. Data was stored on an Eastern Health Encrypted USB key with a password known only by the investigators.

2.2.4 Eastern Cooperative Oncology Group Performance Status

The ECOG Performance Status is a scale developed by the Eastern Cooperative Oncology Group, first published in 1982, comprising six grades, labelled zero to five, with zero meaning a patient is fully active and able to perform all tasks they would have prior to their disease state without any limitations, and five indicating that the patient is dead. The levels in between denote variable levels of disability; one indicates restriction only with strenuous activity; two indicates that the patient is ambulatory and capable of self-care, and active >50% of the time during waking hours, but unable to work; three indicates that the patient is confined to their bed or chair for >50% of waking hours and only capable of limited self-care; four representing the highest level of disability excluding death, where the patient is unable to care for themselves at all and confined to their bed or chair. This scale is frequently used in the setting of clinical trials as a means of characterizing and quantifying patient disability in the study.[140,192]

2.2.5 Karnofsky Performance Status

Another tool used to a patient's performance status is the KPS. It was first published in 1949.[193] The KPS uses an 11-point scale; 100% on the KPS would indicate that the patient had a completely normal functional status, no complaints and no evidence of disease, whereas a score of 0% would indicate that the patient was dead. The rest of the scale is divided into increments of 10%, with a lower number indicating a greater disturbance in the patient's functional status. Similar to the ECOG Performance Status, this scale is frequently in clinical trials to describe disability levels aiding researchers in their subgroup analyses of patients with varying degrees of disability, though the KPS is also used as a decision tool when considering treatment for patients. Péus et al. compare the KPS with the ECOG Performance Status and note that the literature points to neither scale offering a substantial advantage over the other; however, the ECOG Performance Status could potentially lead to oversimplification as the categories are more limited.[137,194]

2.2.6 Charlson Comorbidity Index

The CCI is a tool developed in 1987 by Charlson et al. to predict mortality by assigning weights to specific comorbidities and generating an overall score. It is used widely in the field of health research to measure the burden of disease in individuals. It has been validated in many settings, including cancer,[195] stroke,[196] kidney disease,[197] heart failure,[198] cirrhosis,[199] and critical illness.[200,201] Development of the tool began in 1984 when the authors reviewed 559 medical charts of hospitalized patients admitted to the medicine service and took note of all comorbidities present in these patients. The association of these comorbidities was investigated with reference to one-year all-cause mortality, and 17 of these comorbidities were found to have a statistically

significant association. They assigned a weighted score to each condition based on the relative risk of one-year mortality associated with the particular condition. It was initially validated in the setting of breast cancer, and found to have a strong ability to predict mortality.[202]

Recognizing the significant advances in medicine and medical technology since 1984, Quan et al. revaluated the relative weights assigned to each of the comorbidities using more recent mortality data. They analyzed the data from medical charts and death certificates of all patients discharged from hospitals in the Calgary Health Region (Calgary, Alberta, Canada) in 2004, for a total of 55,929 patient records. They were able to narrow the list to include 12 comorbidities of interest rather than the original 17 identified by Charlson and his team. They validated their results using hospital discharge data extracted from 6 countries.[203] A 2005 study by Sorror et al. discussed their use of the CCI in the prediction of outcomes for patients undergoing allogeneic HSCT in a previous study but noted that very few patients had scores of at least one; therefore, the tool was not particularly helpful in distinguishing those with significant comorbidities from those without. These researchers developed a more sensitive tool that captured 62% of patients with a score at least one, rather than the previously measured 12% using the CCI. Most comorbidities used in this index are the same as those used in the CCI, they simply have much more specific definitions (i.e. CCI defines mild pulmonary disease as "dyspnea on moderate activity," while the new index named the Hematopoietic Cell Transplant Comorbidity Index (HCT-CI) defines the same category as "dyspnea on moderate activity or DL_{CO} and/or FEV_1 of 81-90%.") While this comorbidity index may be superior, we will use the CCI as it is the comorbidity assessment tool used at our centre.

2.3 Sample Size and Data Analysis

For our primary outcome, we used Time-To-Event analysis (Cox regression) to determine the time to engraftment of each cell line (neutrophils and platelets) with respect to post-thaw viable CD34+ cell count. To determine whether post-thaw viable CD34+ cell count correlates to pre-freeze viable CD34+ cell count, and the rate of platelet transfusion, we used linear regression analysis. We used binary logistic regression analysis to determine whether post-thaw viable CD34+ cell count was correlated to the need for ICU admission or the presence of infection. Statistical analysis was performed using IBM SPSS Statistics software, version 27.[204]

We estimated we could include approximately 100 patients in our study, based on an estimate by a faculty member of the hematology department at the Health Sciences Centre with knowledge of the hospital's typical patient volume. We hypothesized that we would be able to obtain a statistically significant result as a 2008 study by Lee et al. attempted to answer a similar question with a sample size of approximately one third of our estimated sample size (36 patients) at the Yonsei University Medical Center in Seoul, South Korea. The authors of this study investigated whether quantification of post-thaw viable CD34+ cell count was a better representation of graft contents than post-thaw total CD34+ cell count or pre-freeze CD34+ cell count.[205]

Chapter 3: Results

3.1 Patient Characteristics

Through our inclusion criteria, we identified 89 patients who had received an auto-HSCT at the Health Sciences Centre in St. John's, NL between January 1, 2014 and August 31, 2019. Of these patients, 88 were diagnosed with MM or lymphoma, and 1 had a germ cell tumour. The single patient receiving auto-HSCT for a germ cell tumour was excluded, as this study aimed to investigate the use of auto-HSCT in hematological malignancy. Of the remaining 88 patients, 40 (45.5%) had a diagnosis of MM and 48 (54.5%) had a diagnosis of lymphoma. Patients diagnosed with MM underwent a high-dose Melphalan conditioning regimen, while 46 of the 48 patients (95.8%) with lymphoma received the BEAM protocol. One patient in the lymphoma category received the BuMelTT protocol, and 1 patient received the R-BuMelTT protocol. The latter two patients were treated with a different conditioning regimen due to the CNS involvement of their lymphoma. This is represented in the flow diagram seen in Figure 3.1

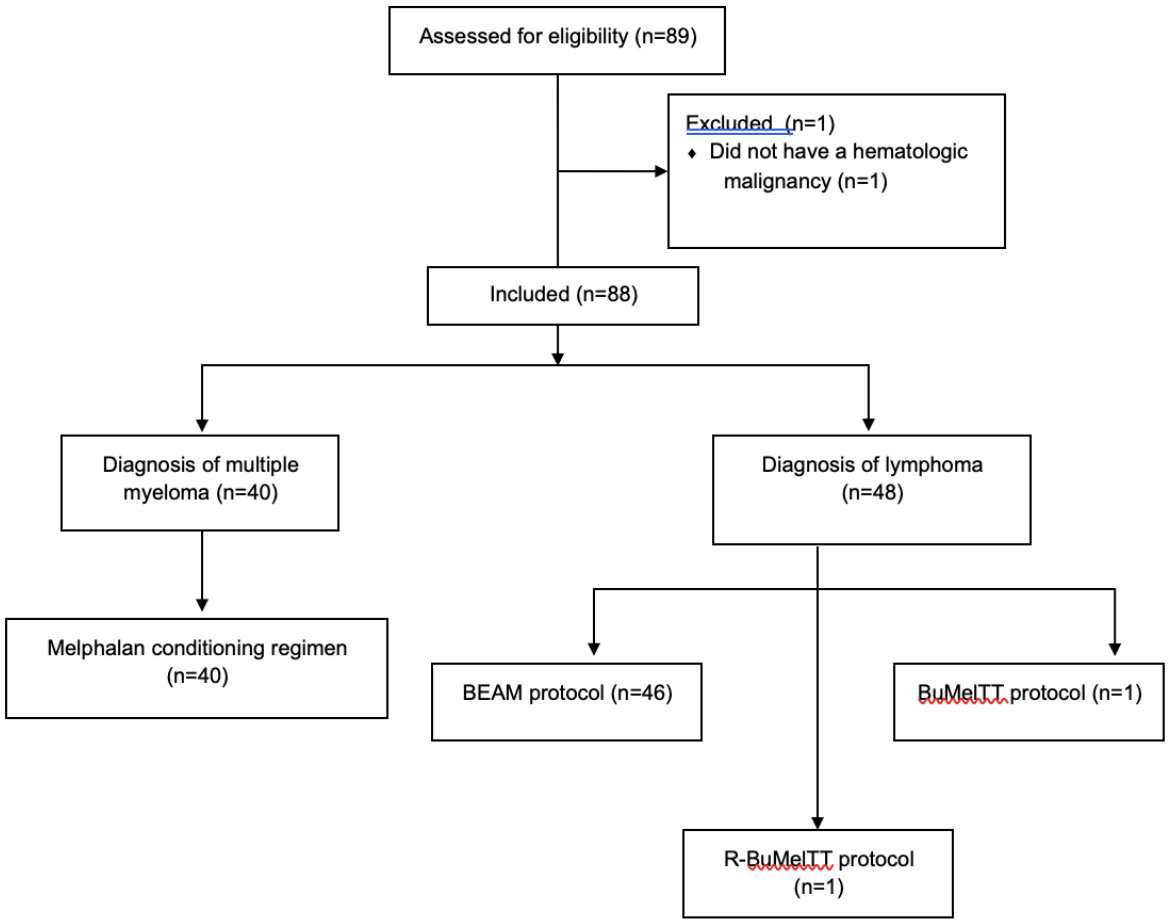

Figure 3.1 Patient Flow Diagram

The average age of patients included in the study was 57.0 (SD=11.3) ranging from 19 to 73 years, with a median age of 59 years. Five patients were <40 years old (5.7%), 12 from 40-49 years (13.6%), 30 from 50-59 years (34.1%), 35 from 60-69 years (39.8%) and six were ≥70 years (6.8%). Fifty-three (60.2%) patients were biologically male and 35 (39.8%) were biologically female. Patient demographics are summarized in Table 3.1

Characteristic	Frequency
Total patients	88
Male	53 (60.2%)
Female	35 (39.8%)
Age (in years)	
Mean	57.03(SD=11.284)
Median	59
Range	19-73
Malignancy	
Lymphoma	40 (45.5%)
MM	48 (54.5%)
ECOG	
0	18 (20.4%)
0.5	1 (1.1%)
1	4 (4.5%)
Karnofsky Score	
70	2 (2.3%)
75	3 (3.4%)
80	34 (38.6)
85	5 (5.6%)
90	17 (19.3%)
95	1 (1.1%)

Table 3.1 Patient Demographics

We initially planned to collect ECOG Performance Status[140] as a measure of functional status, though we were only able to extract data for 23 of the 88 patients (26.1%), as the other 65 patients did not have an ECOG score documented in their medical chart. We then decided to use a comparable measure, the KPS[193] and were able to obtain data for 62 of the 88 patients (70.5%) in the study. The KPS ranged from 70% to 95% in our patient population. Five (5.7%) patients

had scores ranging from 70-79%, 39 (44.3%) patients ranging from 80-89% and 18 (20.5%) had scores of ≥90%. The mean score for the group was 82.8 (SD=5.5).

Using the original version of the CCI, all patients had scores ranging from two to four. Seventy-three (83.0%) patients had a score of two, 11 (12.5%) a score of three, and 4 (4.5%) a score of four.

CCI Score	# of patients (%)
1	0
2	73 (83.0%)
3	11 (12.5%)
4	4 (4.5%)

Table 3.2 Charlson Comorbidity Index

The comorbidities within our patient population are described in Table 3.3

Comorbidity	# of patients (%)
Myocardial Infarction	1 (1.1%)
Congestive Heart Failure	3 (3.4%)
TIA/stroke	1 (1.1%)
Lung Disease	2 (2.3%)
Diabetes Mellitus	5 (5.7%)
Chronic Kidney Disease	1 (1.1%)
Peptic Ulcer Disease	3 (3.4%)
Rheumatic Disease	2 (2.3%)

Table 3.3 Patient Comorbidities

3.2 Primary Outcomes

3.2.1 Neutrophil Engraftment

We identified the date of neutrophil engraftment for all 88 patients in the study. The average time to platelet engraftment was 11.0 (SD=2.4) days with a median of 14 days and a range of eight to 23 days. Time to neutrophil engraftment as a function of post-thaw viable CD34+ cell count is seen in Figure 3.2. Using the chi-square test, we were able to determine that post-thaw viable CD34+ cell count is a statistically significant positive predictor of time to neutrophil engraftment (HR=0.224, 95% CI=1.087-1.440, p=0.002.) When adjusted for diagnosis, a diagnosis of lymphoma rather than MM was a positive predictor of earlier engraftment of neutrophils (HR=2.65, S.E.=0.229, p=0.001).

Using the proportional hazards model, there was no significant (p=0.054) improvement in model fit when adjusting for time-dependent covariates, suggesting that treating any of the covariates as time-dependent variables in this model is unnecessary.

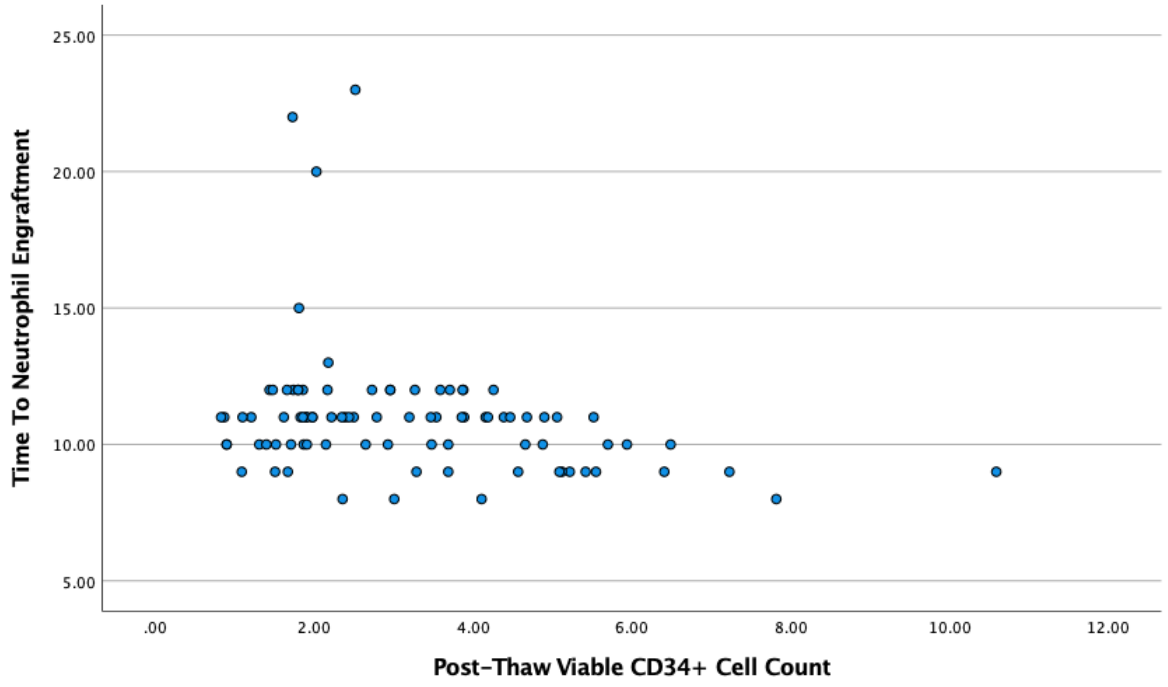

Figure 3.2 Time to Neutrophil Engraftment as a Function of Post-Thaw Viable CD34+ Cell Count

3.2.2 Platelet Engraftment

We determined the day on which platelet engraftment occurred in 87 of our 88 patients; one patient died before achieving platelet engraftment. The average time to neutrophil engraftment was 14.8 days (SD=3.6), with a median time of 11 days and a range of seven to 28 days. Time to platelet engraftment as a function of post-thaw viable CD34+ cell count is seen in Figure 3.3. Using the chi-square test, we determined that post-thaw viable CD34+ cell count is not a statistically significant predictor of engraftment (HR=0.108, 95% CI 0.990-1.255, p=0.83).

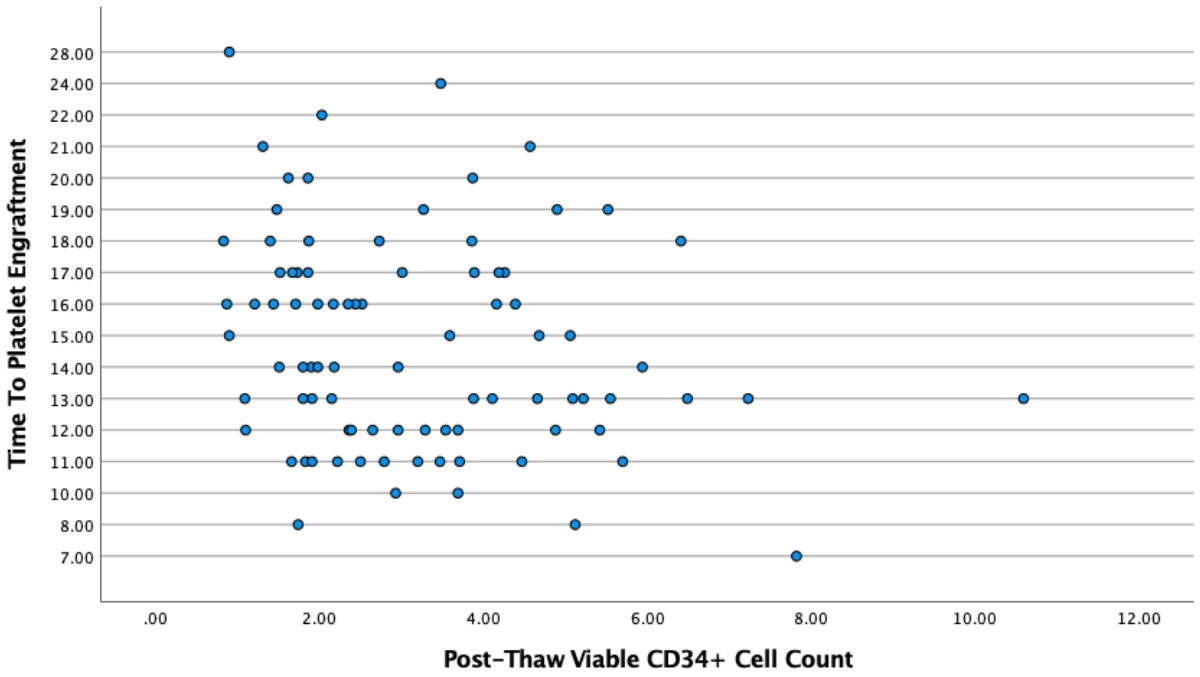

Figure 3.3 Time to Platelet Engraftment as a Function of Post-Thaw Viable CD34+ Cell Count

3.3 Secondary Outcomes

3.3.1 Post-Thaw and Pre-freeze CD34+ Cell Count

Post-thaw viable CD34+ cell count positively correlates with pre-freeze total CD34+ cell count (Pearson's Correlation coefficient=0.744, $p<0.001$.) Post-thaw viable CD34+ cell count as a function of pre-freeze total CD34+ cell count is seen in Figure 3.4

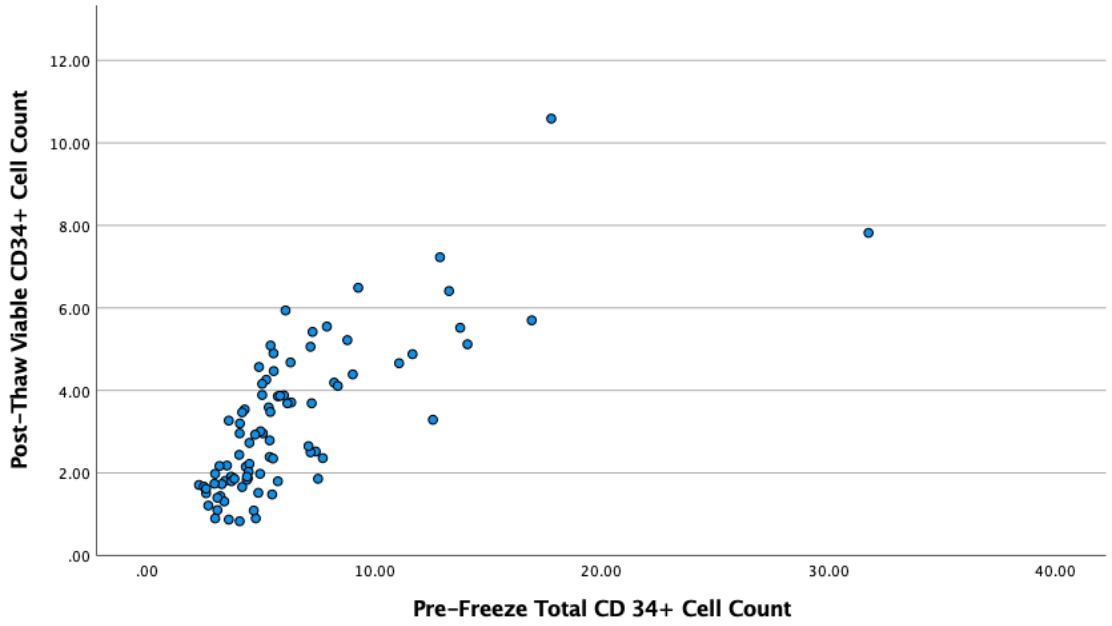

Figure 3.4 Post-Thaw Viable CD34+ Cell Count as a Function of Pre-Freeze Total CD34+ Cell Count

3.3.2 Platelet Transfusion

Seventy-one (80.7%) of the 88 patients required at least one platelet transfusion during the admission following their auto-HSCT. Thirty patients (34.1%) required only a single adult unit of platelets, and 18 patients (20.5%) required two units. Of the remaining 23 patients, 22 (25%) required three to 12 units of platelets, while a single patient required 42 units. These results are pictured in Figure 3.5

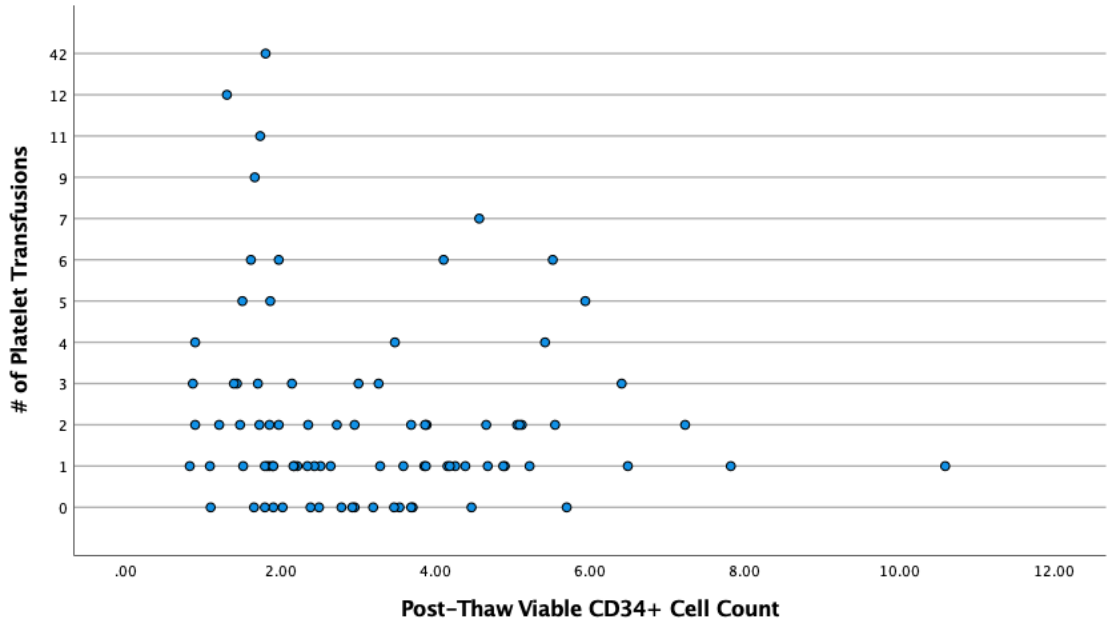

Figure 3.5 Platelet Transfusion as a Function of Post-Thaw Viable CD34+ Cell Count

Using linear regression analysis, we found that the number of units of platelet transfusion required was not significantly correlated with viable CD34+ cell count, (F=1.403, p= 0.239).

3.3.3 Intensive Care Unit Admission

Seven patients (8.0%) had a documented admission to the ICU during the same admission as their auto-HSCT. Using binary logistic regression analysis, we found that CD34+ cell dose cannot significantly predict the need for ICU admission (Chi-square 0.629, p=0.428). The Hosmer and Lemeshow Chi-Square=3.078, p=0.929, demonstrating a good model fit.

3.3.4 Infection

Sixty-six patients (75.0%) demonstrated evidence of infection during their admission (positive culture, suggestion of infection on imaging or culture-negative fever). Twenty-four (27.3%)

patients developed a culture-negative febrile neutropenia. The remaining 42 patients (47.7%) showed evidence of infection through positive culture or on imaging with symptoms consistent with infection documented in the patient's chart. The most common type of infection was a bacteremia, experienced in 28 (31.8%) patients, followed by diarrhea caused by *Clostridioides difficile* in eight (9.1%) patients. Other documented infections included respiratory infection, urinary tract infection, perianal abscess, dental abscess, typhlitis and diarrhea from Cryptosporidium and Enteropathogenic *Escherichia coli* (EPEC).

Type of Infection	# of patients (%)
Infection (All types)	66(75.0%)
Culture-Negative Febrile Neutropenia	24(27.3%)
Culture/Imaging-Positive Infection	42(47.4%)
Bacteremia	28 (31.8%)
Clostridiodes difficile Diarrhea	8(9.1%)
Respiratory Infection	6(6.8%)
Perianal Abscess	3(3.4%)
Urinary Tract Infection	2(2.3%)
EPEC Diarrhea	1(1.1%)
Typhlitis	1(1.1%)
Dental Abscess	1(1.1%)

Table 3.4 Documented Infections

Using binary logistic regression analysis, we found that CD34+ cell dose cannot significantly predict the development of infection (all types pooled) (Chi-square=3.282, p=0.070). We also found that CD34+ cell dose cannot significantly predict the development of a culture- or

imaging-positive infection (Chi-square=3.152,p=0.076), culture-positive infection (Chi-square=3.305, p=0.069), or bacteremia (Chi-square=0.042, p=0.838).

Chapter 4: Discussion

Our study found that higher post-thaw viable CD34+ cell counts were correlated with earlier neutrophil engraftment. A diagnosis of lymphoma rather than MM was also positively correlated with earlier neutrophil engraftment. Weaver et al.'s 1995 analysis of 692 patients treated with high-dose chemotherapy prior to auto-HSCT agreed with our findings on neutrophil engraftment, demonstrating that CD34+ cell count does predict rapidity of neutrophil engraftment through a dose-dependent relationship, though engraftment in this study took an average of nice days (range five to 38 days), whereas our study demonstrated an average engraftment time of 14.7 days (median 11 days, range seven to 28 days).

Our study differed from the 1995 Weaver et al. study in our investigation of the effect of CD34+ cell dose on platelet engraftment as we did not find that higher post-thaw viable CD34+ cell counts were correlated with earlier platelet engraftment, though Weaver et al.'s 1995 analysis did demonstrate a positive correlation of CD34+ cell count with platelet engraftment, demonstrating an average engraftment time of nine days, with a range of four to 53+ days. An explanation for our differing results in terms of both time to neutrophil and to platelet engraftment may be that our study population differed significantly from the 1995 study. A sizeable proportion of our patient population (45.4%) was diagnosed with MM, and the remainder with NHL. In comparison, the Weaver et al. study comprised a population of patients with HL (3%), NHL (17%), stage II-IV breast cancer (71%), ovarian cancer (5%), relapsed sarcoma (3%) or MM (1%). Our study included only hematologic malignancies while the Weaver et al. study comprised mainly breast cancer. The larger sample size of the 1995 study provides more power which may partly explain why the Weaver et al. study demonstrated a statistically significant

correlation between CD34+ cell count and time to platelet engraftment while our study could not. [185] Unfortunately, our study was not able to replicate or on this sample size given that we had a fixed population, incorporating all patients who received an auto-HSCT for hematologic malignancy at our centre since our procedures changes in 2014.

A 2017 study by Martin et al. also investigated the relationship between CD34+ cell dose and platelet engraftment in patients with MM. Their results were consistent with the 1995 Weaver study, finding that increasing CD34+ cell dose predicted earlier platelet engraftment. For each unit increase in CD34+ cell dose, time to platelet engraftment decreased by 2.17 days; however, this was only the case for patients less than 65 years of age. No significant correlation was found between CD34+ cell dose and platelet engraftment in patients with MM aged 65 years or older. Again, the discrepancy in these results and our study may lie in population differences; our population comprised both patients with NHL and MM and the Martin study consisted only of patients with MM.[206] A 2020 study by Lufti et al. supports the theory that engraftment times between patients with MM and NHL are significantly different. Patients with NHL represented 18% of their original 1162-patient cohort, but accounted for 45% of instances of delayed platelet engraftment while patients with MM represented 59% of their patient cohort, but only 29% of instances of delayed platelet engraftment.[207]

A 2011 study by Stiff et al. investigated the relationship between transplanted CD34+ cell dose and its association with long-term platelet count recovery following auto-HSCT in patients with diagnoses of HL (217 patients analyzed) and MM (221 patients analyzed). This study grouped patients to receive one of three CD34+ cell dose levels; 2-4 x 10^6 cells/kg, 4-6 x 10^6 cells/kg, or

>6 x 10^6 cells/kg. There was no significant difference in time to engraftment of neutrophils or platelets among the three dosing levels. This study did find that a linear correlation existed between CD34+ cell dose and a platelet count of ≥150 x 10^9/L at the 100-day mark post auto-HSCT in patients with NHL as well as MM; however, this association persisted to the six and 12-month marks in patients with NHL but not in patients with MM. No significant differences in mean neutrophil counts were found in this study at either the 100-day, six or 12-month marks post auto-HSCT in patients with diagnoses of either NHL or MM.[191]

In our study, post-thaw viable CD34+ cell count was not correlated with the requirement for platelet transfusion, which is consistent with Stiff et al.'s 2011 findings. The 2011 study did not find any significant linear association between CD34+ cell dose across all three dosing categories and the need for platelet transfusion in patients with either NHL or MM. As we investigated a very similar population to this 2011 study, it is gratifying that our results were consistent.[191]

Our study did not find any statistically significant correlation between CD34+ cell dose and the development of any type of infection or ICU admission. Based on our review of the current literature, we did not find other studies which examined these outcomes.

4.1 Limitations

Our study had several limitations. First, we were limited by our sample size. We estimated that we would be able to obtain a sample size of approximately 100 patients, which would, in itself, still be a fairly low number. When we excluded one patient with a non-hematologic malignancy

for whom we had incomplete data, we were left with a sample size of 88 patients. We were able to obtain statistical significance for one of our primary outcomes, namely time to neutrophil engraftment; however, we were not able to obtain statistical significance to show a correlation between CD34+ cell count and time to platelet engraftment, as many preceding studies have. Presumably, as other studies were able to obtain statistical significance for these outcomes, we would have been able to reach a conclusion for or against correlation with a larger sample size; however, this conclusion will need to be deferred as this was unattainable at the time of this study.

Regarding our secondary outcomes, the patient records available to us for this project did not allow us to determine which patients had been admitted to ICU with certainty. At our institution, the patient remains registered under the admitting service for the duration of their entire hospital stay, in this case, hematology, despite whether they remain on the ward being actively managed by the hematology team or if they have been transferred to the ICU. Physician and nursing progress notes often mentioned worsening participant condition and transfer to the ICU or that the participant had just returned to the ward from ICU, though this was not documented universally. Often, the paper chart will be uploaded to the electronic medical record; however, this did not happen in all cases. For this reason, there are likely some inaccuracies within the data set when extracting the data from medical records.

It was also difficult to determine whether a participant had developed a symptomatic infection or not. Unfortunately, vital signs from previous visits are unavailable on our institution's medical records which would have provided information about clinical stability and the potential for

sepsis, using heart rate, blood pressure, respiratory rate, oxygen saturation and temperature. Vital signs and infectious symptoms were not consistently documented in progress notes. For example, at times, we were able to ascertain that bacterial cultures were sent to the laboratory which would presumably be ordered due to clinical suspicion of infection, however, a narrative account of the participant's symptoms was not always available, which may have led to an underestimation of infection rate. In addition to these issues, bacterial cultures may or may not have been performed prior to antibiotic administration, and this was usually not documented. This would reduce the culture's sensitivity and therefore contribute another source of underestimation to our study.

4.2 Future Directions

Our study suggests that CD34+ cell dose measurement may not be a useful quality indicator for autologous stem transplant as we found a correlation between CD34+ cell dose and time to engraftment of neutrophils, though it has significant limitations, as we found no correlation to time to platelet engraftment or to any of our secondary outcomes. For this reason, we may want to consider other methods to determine product adequacy. Adequacy of transplant can also be measured through *in vitro* colony assays, most commonly granulocytes-macrophage colony-forming units (CFU-GM) where the cellular threshold for an acceptable transplant is generally accepted as 1-5 x 10^5 cells/kg of body weight.[4] The EBMT Handbook identifies CFU-GM as the most reliable functional viability indicator, though it also states that there are no guidelines to inform our use of CFU-GM as a quality indicator for auto-HSCT.[9]

A Canadian study from 2000 by Rock et al. identified CFU-GM and CD34+ cell count as the most common methods used to evaluate stem cell content in peripheral apheresis HSCT

products. The multicentre study to compared the reproducibility of CFU cell assays and CD34+ cell count in six transplant centres across the country and found poor interinstitutional reproducibility for both CFUs and CD34+ cell counts. They concluded that a unified interinstitutional quality assurance program was needed, but unfortunately, neither method demonstrated reliable results. They noted that with use of fixed combination of recombinant growth factors, bath testing fetal calf serum, often used for *in vitro* cell expansion in the research setting, and a rigid training program to minimize intra- and inter-staff variability, greater reliability could be achieved,[208] but this would not likely be a feasible approach on a large scale.[209]

CFUs have been studied more recently in slightly different HSCT settings. A 2020 study by Hussein et al. evaluated the role of CFU-GM in the quality assessment of umbilical cord blood in patients receiving auto-HSCT. They found a statistically significant association between post-thaw CFU-GM dose and rapidity of neutrophil and platelet engraftment, with higher doses demonstrating faster engraftment times.[210] They suggested that with further study, this data may be extrapolated to peripheral apheresis to provide a better quality assurance tool that will correlate more closely with actual clinical outcomes.

4.3 Conclusions

Overall, we were not able to generate any compelling evidence that CD34+ cell count has a significant effect on patient outcomes. While we were able to identify that CD34+ cell count was correlated with earlier neutrophil engraftment, we were not able to determine whether this created a meaningful change in the patient's disease course. We would assume that the risk of

infection and potentially the risk of ICU admission would be lower with earlier neutrophil engraftment, but we were unable to show this. We also did not generate any evidence to support a correlation between CD34+ and earlier platelet engraftment, or the necessity for platelet transfusion. A larger study with a similar protocol may be able to demonstrate a statistically significant correlation between CD34+ cell count and clinical outcomes. If not, with a larger sample size, we would still have more robust evidence to suggest that there is indeed no correlation and would support the notion of abandoning this method of quality assessment.

This study did not provide compelling reasons to continue to perform CD34+ for all autologous stem cell transplants. The results of this study have resulted in discussions within our institution to determine how to proceed in terms of quality indicators for auto-HSCT. The evidence supporting the use CFU-GM is currently limited; however, early studies have shown promise. We anticipate further study generating a more robust evidence base in the future, at which time our institution will certainly consider a change in our current quality assurance methods.

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Appendix 1. Conditioning Regimens

BEAM Protocol

The BEAM protocol includes carmustine, etoposide, cytarabine, and melphalan and is administered to patients receiving auto-HSCT for lymphoma at our facility. As per Eastern Health policy, hydration with D5W, 0.45% normal saline with 20 mmol Potassium Chloride/L at 125 mL/hour and allopurinol 300 mg po daily begins 7 days prior to stem cell infusion and continues for 5 days. The patient also receives ondansetron 8mg po every 8 hours for 9 days, metoclopramide as needed for nausea, 1 dose of aprepitant 125mg po and dexamethasone at a dose determined by the administering physician. The dose of carmustine administered ($300mg/m^2$) is determined by corrected body surface area.

Six days prior to stem cell infusion, the patient receives dexamethasone at a dose and frequency determined by the physician, aprepitant 80 mg po daily for 5 days, as well as cytarabine 200 mg/m^2 and etoposide $100mg/m^2$ each, every 12 hours for 8 doses based on corrected body surface area.

Two days prior to stem cell infusion, the patient receives a dose of dexamethasone as determined by the administering physician and 1 dose of melphalan $140mg/m^2$ based on corrected body surface area. After melphalan infusion, the patient is given a dose of furosemide 20 mg IV, 20% mannitol 250 mL IV, and then hydrated with 1500 mL of normal saline over 3 hours and subsequently normal saline with 40 mmol of potassium chloride/L at 125 mL/hour for a total of 18 hours.

On the day prior to stem cell infusion, the patient receives a dose of dexamethasone at a dose determined by the physician, and then another dose on the day of infusion. On the day of infusion, the patient also begins a 14-day course of fluconazole 50 mg po daily and a 6-month course of acyclovir 800 mg po daily. Doses of dexamethasone are also given on days 1, 2, and 3 post stem cell infusion. On the 5th day post infusion, the patient receives a 5-day course of filgastrim (300mcg subcutaneously for patients weighing ≤70kg, or 480mcg subcutaneously for patients weighing >70kg). The protocol concludes with a year-long course of trimethoprim 160mg/sulfamethoxazole 800 mg twice daily po.

Throughout the protocol, patients are transfused with packed red blood cells and platelets as necessary.

Melphalan Protocol

The melphalan protocol is administered to patients with receiving auto-HSCT for MM at Eastern Health. Two days prior to stem cell infusion, the patient is administered Allopurinol 300 mg po daily for 5 days and begins hydration with D5W and 0.45% normal saline with 20 mmol of potassium chloride/L at 125 mL/hour for a period of 16 hours. On the day prior to stem cell infusion, the patient receives Ondansetron 8 mg every 8 hours for 3 days, metoclopramide as needed for nausea, 1 dose of fosaprepitant 150 mg IV and dexamethasone at a dose determined by the physician. The melphalan is subsequently administered at a dose of 200 mg/m^2 based on corrected body surface area, followed by a dose of furosemide 20 mg IV and a 250 mL IV dose of 20% mannitol. The patient is then hydrated with 1500 mL of normal saline over 3 hours,

followed by normal saline with 40 mmol of potassium chloride/L at 125 mL/hour for a total of 18 hours.

On the day of infusion, the patient also begins a 14-day course of fluconazole 50 mg po daily and a 6-month course of acyclovir 800 mg po daily. Doses of dexamethasone are also given on days 0, 1, 2, and 3 post stem cell infusion. On the 5th day post infusion, the patient receives a 5-day course of filgastrim (300mcg subcutaneously for patients weighing ≤70kg, or 480mcg subcutaneously for patients weighing >70kg). The protocol concludes with a year-long course of trimethoprim 160mg/sulfamethoxazole 800 mg twice daily po starting 21 days post-infusion.

Throughout the protocol, patients are transfused with packed red blood cells and platelets as necessary.

Thiotepa Protocol

The thiotepa protocol is indicated for patients receiving auto-HSCT for lymphoma with central nervous system involvement at Eastern Health. It involves the use of rituximab, thiotepa, busulfan and melphalan. The protocol begins 7 days prior to stem cell infusion with the patient starts an 8-day course of allopurinol 300mg po daily and is given metoclopramide as needed for nausea. Prior to administering the rituximab, the patient receives doses of dexamethasone 20 mg IV, acetaminophen 650 mg po and diphenhydramine 50 mg po. Next, the rituximab infusion (375mg/m^2 of corrected body surface area) is started with careful monitoring of the patient for potential adverse reaction including flushing, rigors, rash, pruritis, vomiting, chest pain or any other acute causes of distress. Should an adverse reaction occur, the infusion is stopped until

symptoms subside, and the continued at a slower rate. Physicians may administer doses of meperidine, diphenhydramine and acetaminophen as needed to counteract adverse reactions during the rituximab infusion. On the evening after this infusion, the patient is hydrated with a solution of 5% dextrose and 0.45% normal saline with 20 mmol of potassium chloride and 500 mg of magnesium sulfate/L at a rate of 125 mL/hour for 1 total of 12 hours.

On the 6th day prior to stem cell infusion, the patient begins an 8-day course of ondansetron 8mg po every 8 hours and receives a 250mg/m^2 dose of Thiotepa, calculated based on corrected body surface area. The same thiotepa infusion is administered the following day, with a 4-day course of lorazepam 1mg, orally, 4 times daily beginning that evening.

On the 4th day prior to stem cell infusion, the patient starts a 4-day course of dexamethasone 20mg po or IV once daily and a 3-day course of busulfan (3.2mg/kg of ideal body weight IV) once daily. On the day before stem cell infusion, the patient receives a 1mg sublingual dose of lorazepam in the morning, followed by a 100mg/m^2 IV (based on corrected body surface area) dose of melphalan. Next, the patient is administered doses of furosemide 20 mg IV and 250 mL of 20% mannitol. Hydration begins with 1500 mL IV of normal saline over 3 hours followed by 18 hours of normal saline with 40 mmol potassium chloride/L at 125 mL/hour.

On the day of stem cell infusion, the patient receives an 8mg IV dose of dexamethasone prior to stem cell infusion. They also begin a 14-day course of fluconazole 50 mg po once daily and a 6-month course of acyclovir 800 mg po once daily. On days 1, 2, 3, and 4 post-infusion, the patient receives doses of dexamethasone as determined by the physician. On the 5th day post infusion,

the patient receives a 5-day course of filgastrim (300mcg subcutaneously for patients weighing ≤70kg, or 480mcg subcutaneously for patients weighing >70kg). The protocol concludes with a 6-month course of trimethoprim 160mg/sulfamethoxazole 800 mg twice daily po starting 21 days post-infusion.

Appendix 2. Collection Procedures

The procedure for collection of stem cells is outlined in the Eastern Health Standard Operating Procedure "Collection of Stem Cells using Continuous Mononuclear Cell Collection Procedure," version 1.0. Prior to the collection itself, samples of blood are drawn and sent for complete blood count, CD34+ cell count, ionized calcium, urea, creatinine, electrolytes and type and screen. The patient's weight, height and vital signs are then measured and recorded. A dose of filgrastim is then administered as per the physician's orders, if indicated. Prior to collection, the results of the complete blood count and CD34+ cell count must be returned and be determined adequate to proceed. The white blood cell count must be $>1.0 \times 10^9$ cells/L, and the CD34+ cell count must be ≥5.0 cells/mcL in order to proceed with apheresis. If the CD34+ cell count does not meet this threshold, no apheresis takes place on that day, the patient is given a dose of filgrastim, and a dose of plerixafor is ordered for that evening. The next morning, the CBC is repeated and should the count remain <5.0 cells/mcL, apheresis is cancelled and filgrastim and plerixafor are discontinued.

If, on the planned day of collection, CD34+ cell count is ≥5.0 cells/mcL, then apheresis may proceed, and the patient will be given a dose of filgrastim. Once the stem cells are collected, the product is sent for CD34+ cell count; if the CD34+ cell count is $<2.5 \times 10^6$/Kg, a dose of plerixafor is ordered for that evening, and may be given up to 4 times until collection is complete. If CD4+ cell count is ≥2.5 x 10, continue collection until the target is reached. A flow chart of the procedure is seen in Figure 1A.

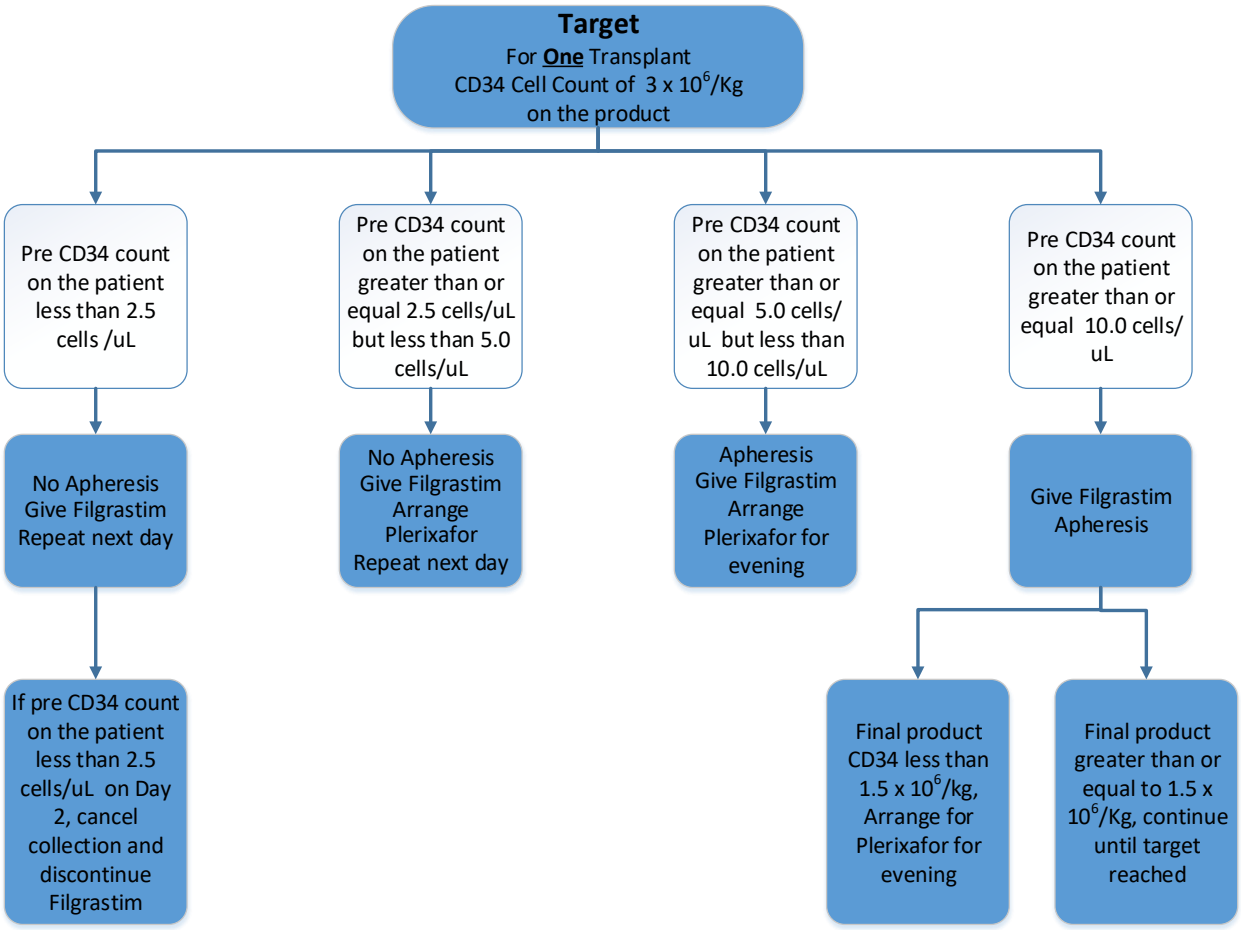

Figure 1A. Flow diagram for Procedure according to CD34+ Cell Count of Product

The Spectra Optia Apheresis System is used to collect the stem cells. The machine is assembled and the tubing primed using a solution containing 4g of calcium chloride in 500 mL of normal saline.

The patient's clamped double-lumen central line is connected to the access line in a sterile fashion, and unclamped once connected. The central line is then clamped again, and the second lumen is attached in a sterile fashion to the return line of the apheresis machine, and unclamped once connected. The patient's vital signs are monitored throughout the apheresis procedure whenever clinically indicated. The machine is turned on and the calcium chloride infusion is

stared at a rate of 65 mL/hr. The blood is collected at a flow rate of 45-80 mL/minute. Fifteen mL of blood is processed through machine during the collection session with a minimum of 150 mL collected. An anticoagulant is given at a rate of 0.8-1.2 mL/minute/L of total blood volume. Once the collection is complete, a 1 mL aliquot is taken from each collection bag, and the access line is clamped, sealed and cut. The aliquots are sent for bacterial culture. Vital signs are checked once more and if stable, the patient is disconnected from the apheresis machine, and the central line is flushed. The CD34+ cell count is measured through flow cytometry and a member of the team will determine whether further collections are needed on subsequent days. Typically, collection takes 4-5 hours to complete.

Appendix 3. Cryopreservation

The procedure for cryopreservation of the hematopoietic stem cells is detailed in the Standard Operating Procedure Number 7179, Version 3.0 belonging to Eastern Health. This policy came into effect on July 4, 2014. Cryopreservation of hematopoietic progenitor cells harvested for auto-HSCT is performed as per institutional policy (Division of Hematology, Eastern Health). When the product arrives in the lab, its weight is recorded, and it is subsequently stored in the refrigerator. Policy states that the product may be stored overnight at 4^{o}C in a monitored refrigerator for processing the following morning without undergoing volume reduction, should the technologists be unable to process the product immediately; otherwise, processing of the product begins at this time.

Infectious Disease Testing

Sterility testing is first performed on the patient's peripheral blood within 30 days prior to the date of collection. Sterility is checked by injecting a 1 mL volume of cells into an aerobic and anaerobic culture bottle for a total incubation period of 28 days. The cell product is also tested for anti-CMV IgG and IgM, Hepatitis A, Hepatitis B, Hepatitis C, Syphilis, HIV and human lymphotropic virus (HTLV) I & II, or as requested by the transplant physician. An aliquot from each day of donor serum collection is sent to the laboratory for infectious disease testing. The apheresis nurse delivers the donor serum to the flow lab each morning of collection. Samples must be spun in the centrifuge within 2 hours of collection, then transferred to a cryovial, inserted in a storage container and stored in the -85^{o}C freezer.

Product Acquisition and Testing

The apheresis product is collected from the donor and transporter in specialized transport containers to the processing laboratory. The apheresis product is contained within Ziploc bags for transport. On arrival to the laboratory, the receipt time and volume of the cells is recorded.

The product is mixed by gently rocking the collection bag back and forth. The collected stem cell product is weighed after a 1.5 mL aliquot is removed. 0.5 mL of the 1.5 mL aliquot is set aside for nucleated cell count (NCC; defined as the total number of white blood cells present in the cellular product) performed with hematology analyzers, 0.5 mL for CD34+ cell count and viability through flow cytometry and 0.5 mL is stored in a cryovial in the 2-8^{o}C refrigerator. This last sample can be discarded after 72 hours if no further testing is needed.

Volume Reduction

Air is injected into the apheresis collection bag through a 50 mL syringe 8-10 times. The cell product is transferred into a 300 mL transfer pack (for cell volumes ≤375 mL) or a 600 mL transfer pack (for volumes ≥375 mL by connecting the collection bag to a transfer pack via a luer lock and hanging the collection bag upside down, allowing the cells to drain into the transfer pack. Once all cells have been transferred, the collection bag is squeezed to force air into the transfer pack. The tubing connecting the collection bag to the transfer pack is clamped using a hemostat clamp and subsequently sealed. The collection bag is then discarded and the transfer bag containing the stem cells is then weighed and recorded. If the weight of the bag differs >10% of the original bag volume, an investigation is initiated to resolve the discrepancy. Once the weight of bag is determined a pre-processing total nucleated cell (TNC) count is calculated using

the following formula: TNC x 10^{10} = [(weight/volume of hematopoietic progenitor cells) x (NCC x 10^{7})]/1000.

The bag of cells is placed in the centrifuge along with a pre-made balanced bag and spun at 3000 rpm for 5 minutes. The spun bag of cells is removed from the centrifuge carefully as to not disturb the buffy coat layer and placed on the plasma extractor. The lever of the plasma extractor is released, and the supernatant plasma is expressed off the cell product. The line connecting the bag of cells to the transfer pack is clamped, and the product is suspended to examine for clumping or clotting. Should significant clumping or clotting be present, the cells must be filtered. To filter, the bag of cells is attached to a filter via a spike port and roller clamp attached to a new transfer pack. The line connecting the filter pack and bag of cells is clamped with a hemostat clamp and the seal is heated prior to filtration. The bag of cells is hung, and roller clamp opened allowing the cells to move through the filter into the new transfer pack. Once all of the cells are in the new transfer pack, the roller clamp is closed, and the sample is mixed. A 0.5 mL sample is taken from the cellular product via a sampling spike for NCC using hematology analyzers, once filtration is complete, if necessary, and the cell count is recorded.

The cell product must be frozen at a concentration of ≤5 x 10^{8} cells/mL. If NCC prior to the addition of a 20% DMSO solution is >1 x 10^{9} cells/mL, then the cell product must be diluted using autologous plasma that was previously removed from the product during the volume reduction process. If this step is necessary, then the cell count is repeated to ensure the concentration is <1 x 10^{9} cells/mL. If the NCC is not within 75-110% of original count, counts are repeated and potential sources of error investigated. Once cell counts are confirmed to be

accurate, the cell product is weighed, and the actual total nucleated cell count (TNC- a measure of total nucleated cells volume of the cellular product) post volume reduction (TNC x 10^{10} = [(weight/cell volume) x (NCC x 10^7)] / 1000 and the TNC % recovery (processed TNC % recovery = (processed TNC/unprocessed TNC) x 100) are calculated. The cells are then refrigerated. The TNC % recovery is used to calculate the total number of CD34+ cells with the following formula: CD34+ x 10^6 /kg = {[(CD34+ per mcmL x initial lab volume)/1000)] x TNC % recovery}/ patient weight in kg.

Cryoprotectant Solution Preparation

The 2 reagents involved in cryopreservation are plasma-lyte A and DMSO. The volumes of the reagents needed to prepare a 20% DMSO solution in Plasma-lyte A to a volume equal to the post-reduction cell volume are calculated. The calculated volume of plasma-lyte A is aspirated into a 50 mL syringe and combined with the cell product in a new transfer pack. A second 50 mL syringe is used to aspirate the calculated DMSO from the bottle. The syringe containing the DMSO is attached to the transfer pack, and plasma-lyte-A is aspirated back into the DMSO-filled syringe, and then the mixture is plunged into the transfer pack. The transfer pack containing the stem cells is then removed from the fridge and combined with the 20% DMSO solution via luer lock, clamped and placed in the 2-8ºC refrigerator until the product reaches its start temperature (2ºC). The cryopreservative and cells do not mix (eg. the line is not unclamped) until the freezer has reached its starting temperature and is ensured to be functioning properly.

Controlled Freeze

A liquid nitrogen supply tank is attached to the control rate freezer with a psi of around 22 which allows liquid nitrogen to run into the freezer once the tank valve is open and freezer is turned on a profile started.

Preparation and Sampling of the Cellular Product

The cooled cells and the 20% DMSO solution are removed from the refrigerator. The DMSO solution is suspended from a hook and attached to the cell bag through a transfer line. The line is unclamped at this time, and the DMSO solution is allowed to transfer into the bag of cells, which are then gently rocked to mix the solution as it is added. Once the solution has been completed transferred, the pack is sealed and cut with scissors. Once the cells and solution are thoroughly mixed, a 1mL sample is withdrawn and sent for culture. Next, a further 3 mL are withdrawn from the cellular product to place 1 mL in each of 3 cryovials. Two of these vials are for quality control, and the third for post-thaw testing. The cells are then transferred to cryostore bags via 50 mL syringe and the volume of each bag is recorded. The air bubbles are removed from each bag of cells with an empty syringe, then the bag is sealed and tubing is discarded. A 2 mL sample is removed for sterility testing; 1 mL is placed in each and aerobic and anerobic blood culture bottle.

The component bags are laid on cool packs and cassettes from the refrigerator on top of a sterile towel and the volume of each bag is recorded. The sterility samples removed for culture are sent to the laboratory for analysis and the cassettes and cryovials are brought to the freezer room for cryopreservation. Component bags and cassettes are labelled during this step.

Cryopreservation

The cassettes and cryovials are gently mixed. The cryovials are placed on the bottom of the freezer in the vial holder and the cassettes full of stem cells are placed upright in the racks. Once the temperature of the freezer has stabilized (approximately 5 minutes), the controlled freeze is started and the time is recorded. The following parameters are set for the freeze: -1°C/minute until reaching a temperature of -60°C, then -5°C/minute until reaching a temperature of -90°C. Once the full freeze program is complete, the cassettes and cryovials are removed from the freezer and transferred to a liquid nitrogen vapor phase freezer.

Preparation for Infusion

The temperature of the water bath is verified as being between 36°C and 40°C and adjusted if necessary. Next, the cassettes are removed from the dry shipper and placed in sterile Ziploc bags on a sterile towel on the reinfusion cart and as much air as possible is removed from the Ziploc bags before sealing them. The bags are subsequently immersed in the water bath and the timer is started. The bags are gently agitated and kneaded while in the water bath, and then removed once they have thawed and no more ice is visible, which takes approximately 2 minutes. The component bags are removed from the Ziploc bags and inspected for any breaks in the bag or any remaining ice in the product. If ice remains, the bag is gently massaged until the ice is no longer visible. The thawing time is recorded. This process is repeated for all component bags, 1 at a time.

Appendix 4. Thawing and Reinfusion

The procedure for the reinfusion of the stem cells into the patient is described the Eastern Health Standard Operating Procedure "Reinfusion of Cryopreserved Blood Stem Cells" version 2.0. Reinfusion takes place as ordered by the physician but requires a minimum period post-conditioning regimen of 72 hours after carboplatinum, 48 hours after cyclophosphamide and/or etoposide, or 24 hours after any other type of chemotherapy.

Prior to the commencement of the reinfusion process, a physician must be available. The patient's central line is then flushed to ensure patency. The IV line is primed with normal saline and connected to 1 lumen of the patient's central line. Pre-medications are administered at this time in normal saline as ordered by the physician. Vitals signs are then measured.

The procedure for thawing hematopoietic stem cells once they have been cryopreserved is detailed in Eastern Health's Standard Operating Procedure number 7192, version 2.0. This policy came into effect on May 31, 2017. The Dry Shipper is filled with liquid nitrogen at least 1-2 days prior to the date of infusion in anticipation of the thawing procedure.

A water bath is placed on the reinfusion cart and filled with approximately 4 L of deionized and purified water and brought to the patient's hospital room 1 hour prior to reinfusion. The water bath is plugged into an emergency outlet through an extension cord and water bath turned on and pre-set temperature for 36-40^{o}C. It takes approximately 50 minutes for the water bath to reach this pre-set temperature.

The temperature of the dry shipper is checked prior to removing the component bags from the liquid nitrogen vapor phase storage freezers, which should be maintained <-120^{o}C. The component bags are removed from the freezer and kept in the metal cassettes and placed in the dry shipper.

Once the first bag of stem cells has been completely thawed, the clamped bag is connected to the tubing through a port using aseptic technique. Before releasing the clamp, the line to the normal saline bag is clamped. Once reinfusion begins, each bag must be completely reinfused into the patient within 10 minutes of thawing. Once the first bag has been administered, the second bag is thawed and the procedure is repeated. Vital signs are measured every 5 minutes throughout the infusion process, or more frequently if clinically indicated. Once the infusion is finished, the central line is flushed with normal saline and vital signs are measured every 15 minutes for 1 hour, or until the patient is stable. The patient is also monitored for signs of anaphylaxis. Minor reactions such as nausea, vomiting, restlessness, flushing, feeling of warmth, coughing and chest tightness are commonly observed after the administration of the DMSO solution; the infusion is not stopped for mild or moderate reactions.

Appendix 5: HREB Approval

Research Ethics Office
Suite 200, Eastern Trust Building
95 Bonaventure Avenue
St. John's, NL
A1B 2X5

March 12, 2020

21A Weymouth St.
St. John's, NL
A1B 2B7

Dear Dr Small:

Researcher Portal File # 20201743
Reference # 2020.025

RE: Analysis of clinical outcomes in autologous stem cell transplant in relation to CD34+ cell count

Your application was reviewed by a subcommittee under the direction of the HREB and the following decision was rendered:

X	Approval
	Approval subject to changes
	Rejection

Ethics approval is granted for one year effective March 12, 2020. This ethics approval will be reported to the board at the next scheduled HREB meeting.

This is to confirm that the HREB reviewed and approved or acknowledged the following documents (as indicated):

- Application, approved
- Research proposal, approved
- Signed Variable request form, approved
- List of Variables, approved

Please note the following:

- This ethics approval will lapse on March 12, 2021. It is your responsibility to ensure that the Ethics Renewal form is submitted prior to the renewal date.
- This is your ethics approval only. Organizational approval may also be required. It is your responsibility to seek the necessary organizational approvals.
- Modifications of the study are not permitted without prior approval from the HREB. Request for modification to the study must be outlined on the relevant Event Form available on the Researcher Portal website.
- Though this research has received HREB approval, you are responsible for the ethical conduct of this research.
- If you have any questions please contact info@hrea.ca or 709 777 6974.

The HREB operates according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS2), ICH Guidance E6: Good Clinical Practice Guidelines (GCP), the Health Research Ethics Authority Act (HREA Act) and applicable laws and regulations.

We wish you every success with your study.

Sincerely,

Health Research Ethics Board