# INTRODUCTION

Hematopoietic stem cells (HSCs), mainly derived from the blood or bone marrow, are used for transplantation with a goal that various diseases can be cured. Engraftment, which refers to the introduction and immunological acceptance of bone marrow introduced during a bone marrow transplant, is preferred to be done using hematopoietic stem cells because of their ability to differentiate into a variety of different cells. Differentiation lowers the chances of rejection. T-cells, Angiotensin II and radiation have positive effects on engraftment. Furthermore, cloning stem cells generates histocompatible cells capable of longterm multilineage engraftment ***(Yoshiokam et al., 2005).***

The development of a comptent immune system follow hematopoietic cell transplantation (HCT) is necessary for a clinically successful transplant. Without immune reconstitution HCT recepients are at an increased risk of infections with opportunistic organisms (viruses, bacteria, fungal and protozoa) and possibly neoplastic relapse. Regardless of the factors that influence immune reconstitution such as HSC source, Graft-Versus-Host disease (GVHD) and its treatment and the preparative regimen, immune reconstitution follows a general pattern. In the recepients of ablative therapy followed by HCT, the recepients` pre-existing lymphoid immunity is eliminated by the immune-suppressive and chemotherapeutic agents used

in the preparatory regimen. Lymphoid immunity following HCT comes from a recapitation of normal lymphoid immunity derived from the newly engrafted HSCs and the mature lymphoid elements present in the graft (both T and B lymphocytes) ***(Robertson and Kenneth, 2009).***

CXCR4(also known as CD184) is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12), a molecule endowed with potent chemotactic activity for lymphocytes. CXCR4's ligand SDF-1 is known to be important in hematopoietic stem cell homing to the bone marrow and in hematopoietic stem cell quiescence ***(Saini et al., 2010).***

There is convincing evidence that the interaction of SDF-1 with its cognate receptor CXCR4 generates signals that regulate HSC trafficking in bone marrow. SDF-1 is a chemokine expressed in the bone marrow by the stromal cells and bone marrow osteoblasts. SDF-1 is a potent chemoattractant agent for HSCs and has been shown to regulate HSC survival, adhesion and cell-cycle status ***(Thomas and John, 2009).***

So, SDF-1/CXCR4 interactions participate in controlling the retention of hematopoietic cells within the BM and their release into the circulation. CXCR4 receptor expression is required for the retention of granulocyte precursors and mature neutrophils within the bone marrow, and disruption of the SDF-1/CXCR4 axis in the bone marrow results in the mobilization of myeloid lineage cells to the peripheral circulation ***(Hyun et al., 2006).***

Interruption of SDF-1/CXCR4 signaling/interaction represents a key step in HSC mobilization by G-CSF. SDF-1 levels in the bone marrow declines sharply during G-CSF mobilization. The decrease in SDF-1 levels in the bone marrow after treatment with G-CSF correlates well with the magnitude of HSC mobilization ***(Thomas and John, 2009).***

CD26 (dipeptidylpeptidase IV [DPPIV]) is a membrane-bound extracellular peptidase that cleaves dipeptides from the N-terminus of polypeptide chains after a proline or an alanine. The N-terminus of chemokines is known to interact with the extracellular portion of chemokine receptors. Consequently, the removal of the N-terminal amino acids results in significant changes in receptor binding and/or functional activity. CD26 only has the ability to cleave chemokines containing the essential N-terminal as CXCL12 ***(Hal and Franklin, 2009)***.

The role of CD26 expression in haematopoietic stem cell transplantation (HSCT) and modulation of stem cell homing and engraftment has been demonstrated in mouse model using human cord blood haematopoietic stem and progenitor cells (HSPCs) and mouse HSPCs in utero . It was demonstrated that inhibition or deletion of CD26 on donor cells enhanced short-term engraftment, competitive repopulation, secondary transplantation and mouse survival. CD26 expression on leukapheresis stem cell which are collected from cancer patients selected for autologous transplantation or from their allogeneic related healthy donors has a role in their harvest and engraftment ***(Prabhash et al., 2010).***

CD26 has been shown to have selectivity for SDF-1/CXCL12. The SDF-1receptor expressed on hematopoietic cells, some of which also express high levels of CD26. Recent in vitro studies have demonstrated that cleavage of SDF-1 by CD26 results in a loss of its chemotactic effect on primitive hematopoietic cells and that inhibition of this action results in increased SDF-1induced migration ***(William et al., 2006).***

# AIM OF THE STUDY

*The aim of this study is to:*

- Assess successful engraftment both in allogeneic and autologous transplant patients using CD184 (CXCR4) and CD26.
- Correlate engraftment with CD184 (CXCR4) and CD26 in stem cell harvest.

Chapter (1)

# AN OVERVIEW ON HSCT

Transplantation of hematopoietic cell grafts containing pluripotent HSCs after myeloablative or nonmyeloablative conditioning regimens reconstitutes immunohematopoiesis. Sites from which HSCs can be harvested and then used for transplantation include bone marrow, peripheral blood, and umbilical cord. Patients may serve as their own donors (autologous) or may receive HSCs from other individuals (related or unrelated). Hematopoietic cell transplantation (HCT) is done for a variety of therapeutic indications: **(a)** to support hematopoiesis after myeloablative doses of total body irradiation (TBI) and chemotherapy, **(b)** to establish a graft versus leukemia or tumor (GVL or GVT) reaction, or **(c)** to replace diseased tissues of hematologic or immunologic origins. The advances in supportive care after transplantation have resulted in HCT being a therapeutic modality that can be successful in otherwise fatal diseases ***(Vijayakrishna and Richard, 2009).***

## Historical background:

In the morning of the atomic age, there was optimism that the nuclear genie could heal as well as kill. By the mid-1950s, researchers had cured mice of leukemia by destroying diseased bone marrow with near-lethal doses of radiation, then rescuing them by transplanting healthy marrow. But

experiments on humans were stymied by fears that radiation exposure sufficient to kill the cancer might also kill the patient. Then came the news that six physicists had become ill from radiation exposure during a 1958 nuclear reactor accident in Yugoslavia. One physicist died from especially heavy exposure, and one was relatively unaffected. Dr. Georges Mathé used the remaining four in a radical experiment. He injected bone marrow collected from donors to replace the damaged marrow. For the first time, a human marrow transplant seemed to take, and the physicists survived. It was called the first successful bone marrow transplant not performed on identical twins. In 1963, four years after treating the physicists, Dr. Mathé shook the medical world by announcing that he had cured a patient of leukemia by means of a bone-marrow transplant. In this case, there was no doubt that he had performed the feat of obliterating the patient's stem cells and replacing them with a donor's: the patient's blood type had changed to that of the donor, the first time this had happened. Most important, he demonstrated that stem cells injected in a patient not only heal radiation damage but also fight cancer. The reason is that the donor's cells rule the body of the patient — whose own cells have been devastated — and attack the cancer as a foreign invader. The donor cells, however, can also attack the patient's healthy ones, and vice versa. Preventing these attacks has been a principal concern of transplant science. The leukemia patient died after 20 months, apparently of encephalitis. Judgments vary on whether the patient's survival was long enough for Dr. Mathé to declare success. Dr. Mathé

worked at a time when original research on bone marrow transplants was percolating. A leader was Dr. E. Donnall Thomas, an American whose work was later recognized with a Nobel Prize in Physiology or Medicine. In 1956, he performed the first bone marrow transplant on a leukemia patient using marrow from an identical twin. In 1969, Dr. Thomas led the team that performed the first transplant between people matched by molecular analysis ***(Douglas, 2010).***

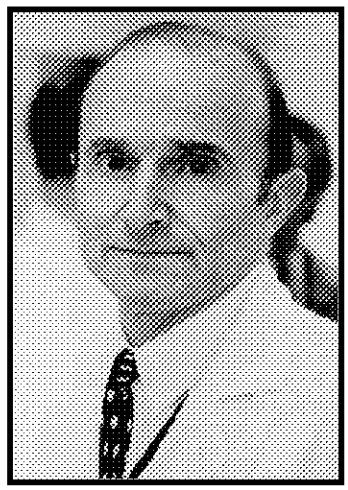

**Figure (1):** Dr. Georges Mathé in 1971***(Douglas, 2010).***

The first physician to perform a successful human bone marrow transplant on a disease other than cancer was Robert A. Good at the University of Minnesota in 1968. The 1968 transplant involved a 5-month-old boy with a profound immunal deficiency that had killed 11 male members of his extended family. Dr. Good led a team that corrected his fatal condition with bone marrow transplanted -- that is, extracted and infused intravenously -- from his 8-year-old sister. The

infant who received the 1968 transplant grew up to healthy adulthood ***(Wolfgang, 2003).***

## Number of procedures:

A total of 50,417 first hematopoietic stem cell transplants were reported as taking place worldwide in 2006, according to a global survey of 1327 centers in 71 countries conducted by the Worldwide Network for Blood and Marrow Transplantation. Of these, 28,901 (57%) were autologous and 21,516 (43%) were allogeneic (11,928 from family donors and 9,588 from unrelated donors). The main indications for transplant were lymphoproliferative disorders (54.5%) and leukemias (33.8%), and the majority took place in either Europe (48%) or the Americas (36%). In 2009, according to the world marrow donor association, stem cell products provided for unrelated transplantation worldwide had increased to 15,399 (3,445 bone marrow donations, 8,162 peripheral blood stem cell donations, and 3,792 cord blood units) ***(Gratwohl et al., 2010)***.

## Indications:

*There are many conditions which are treated with HSCT:*

- ✓ Leukemias: AML, ALL, CML, CLL.
- ✓ Lymphomas: Hodjkin disease, NHL.
- ✓ Plasma cell disorders: MM and related disorders.
- ✓ Solid organs neoplasias: eg: Breast cancer, Ovarian cancer, Lung cancer, Sarcoma, Melanoma, Neuroblastoma.

- ✓ Myelodysplastic syndromes.
- ✓ Severe Aplastic anemia.
- ✓ Autoimmune disease: eg: Systemic lupus erythematosus, Multiple Sclerosis, Systemic Sclerosis.
- ✓ Inherited erythrocyte abnormalities: Sickle cell disease & Thalassemia.
- ✓ Inherited metabolic diseases: eg: Mucopolysaccharidosis.
- ✓ Primary immunodeficiencies: eg: Severe combined immunodeficiencies, Wiskott-Aldrich syndrome, etc.. ***(Chinen and Buckley, 2010).***

**Indications for HSCT in ALL:**

HSCT in ALL should be confined to a small proportion who have low chance of cure with chemotherapy, either in the first complete remission, or second remission or beyond ***(Chi-Kong, 2010).***

**** Indication for HSCT in the first complete remission (CR1):***

Previously, high white blood cell count (WBC) and T-cell ALL were considered as the poor prognostic factors and indication for HSCT in children. With improvement in chemotherapy, chemotherapy outcome in these patients is now approaching the standard risk patients, and high WBC count and T-cell ALL are no more included as indications for HSCT. Cytogenetic abnormalities are found to be predictive of relapse. Philadelphia chromosome (Ph) occurs in 3-5% of all childhood

ALL and is associated with poor prognosis. Ph ALL with poor prednisone response is particularly at high risk of induction failure or relapse. Most transplant centers accept CR1 Ph ALL as indication for transplant, including both related and unrelated HSCT. Recent studies showing that combination of imatinib and intensive chemotherapy can achieve a 3-year disease free survival (DFS) of 80% necessitate redefining need for HSTC in patients with Ph ALL in CR1 ***(Chi-Kong, 2010)***.

Early treatment response is also highly predictive of subsequent relapse. Poor prednisone response (PPR) on day 8 of steroid prephase treatment was found to be a poor prognostic factor. However, intensive chemotherapy can now achieve 60% DFS in patients with PPR only, but patients with other poor prognostic factors should be considered for transplant in CR1. Infant ALL with mixed lineage leukemia (MLL) gene rearrangement and PPR has particularly poor prognosis, and HSCT in CR1 is more acceptable for them. Induction failure after 4-5 weeks of modern intensive induction chemotherapy is another poor prognostic factor. Some of these patients may never enter into CR1 and they do not benefit from HSCT while still having active leukemia. Even if remission is achieved with further intensive chemotherapy, risk of relapse is still high and thus HSCT in CR1 in late responders is also accepted. Another important early response marker is the minimal residual disease (MRD) after induction chemotherapy or early consolidation treatment. Flowcytometry or polymerase chain reaction (PCR) for leukemia specific clone or gene rearrangement is now

included in many clinical trials for risk stratification. High MRD level is associated with high chance of relapse and thus some centers now include MRD as an indication for HSCT in CR1. However, the critical level of MRD for HSCT is variable as it depends on the type of chemotherapy used and also the timing of MRD assessment ***(Chi-Kong, 2010).***

Most transplant centers now accept the following indications for transplant in CR1 in ALL:

(1) Induction failure after 4-5 weeks of standard induction chemotherapy.

(2) High risk Ph+ ALL such as PPR.

(3) High MRD at 1-3 months after start of chemotherapy, although the assessment method and critical level of MRD varies according to study groups.

(4) Infants with MLL rearrangement constitute another group of patients with poor outcome after chemotherapy; however the value of HSCT is controversial ***(Chi-Kong, 2010).***

**** Indication for HSCT in relapsed ALL:***

In the past, relapsed ALL was always considered an indication for HSCT. The BFM studies of treating relapsed ALL patients using uniform protocols identified the risk factors predicting treatment failure. T-cell ALL or Ph ALL with relapse are very difficult to be cured with chemotherapy, therefore HSCT should be considered. Timing and sites of relapse are

other important prognostic factors for DFS in relapsed ALL. Those with early bone marrow relapse within 30 months (especially those within 18 months) from diagnosis have very poor prognosis. HSCT should be arranged in these patients early, as the second remission is not durable and patients may develop second relapse within a short period. For unknown reasons, those with combined bone marrow and extramedullary relapse would fare better with chemotherapy treatment. The MRD level after re-induction treatment is now also included in the risk stratification for HSCT. Patients with late bone marrow relapse have a good chance of cure with second course of chemotherapy if they can achieve a very low MRD after the induction chemotherapy; HSCT may not be indicated for this group of patients. Some patients may have second relapse, then HSCT is always indicated once they can get into another remission. If patients cannot get into complete remission, HSCT is not successful to maintain a long term DFS and should not be considered until a consolidated remission is achieved. Isolated extramedullary relapse is seldom indicated for HSCT, as local therapy such as cranial irradiation with further systemic reinduction chemotherapy is successful to achieve cure in a high percentage of patients ***(Chi-Kong, 2010).***

**Indications for HSCT in AML:**

***AML in CR1:***

AML still has less favorable outcome compared with ALL. With the modern aggressive chemotherapy treatment, the overall DFS is now reported to be 50-60% in recent reports. Studies have now identified a favorable cytogenetic group that can be treated with chemotherapy and achieve a good outcome, namely t(15;17), t(8;21), and inv (6). The DFS of this 'good risk' group is over 60% and most centers do not consider this group as an indication for HSCT in CR1. COG and UKMRC studies confirmed that sibling donor HSCT in favorable cytogenetic group does not confer superior outcome compared with chemotherapy arms. Acute promyelocytic leukemia (APL) now has 80% chance of long-term DFS when transretinoic acid is included as part of the chemotherapy protocol. Even if relapse occurs in APL patients, arsenic trioxide can successfully maintain a prolonged second remission. Thus APL is now seldom included for HSCT in most clinical studies ***(Chi-Kong, 2010).***

For the non-good risk patients, they may be considered for HSCT if HLA-identical sibling donor is available. Early treatment response as determined by blast percentage (15% to 25%) after 2-4 weeks of induction is also an important prognostic factor. Some studies will consider the late remitters or those with slow responses for HSCT in CR1. Unfavorable cytogenetic features such as monosomy 5 and 7 have poor treatment outcome, and HSCT in CR1 is always indicated ***(Chi-Kong, 2010).***

Juvenile myelomonocytic leukemia (JMML) has poor response to intensive chemotherapy, and most centers recommend early HSCT without attempting intensive treatment to achieve remission. A small proportion of JMML patients, especially those without monosomy 7 or abnormal cytogentic features, may remain in long-term remission with mild chemotherapy. Therefore, an initial trial of chemotherapy may be justified in this group of patients ***(Chi-Kong, 2010).***

***Relapsed AML:***

Relapsed AML is always associated with poor prognosis except APL. Most of the relapses occur in bone marrow. It is a common indication for HSCT in most studies. However, a small percentage of patients with late relapse with CR1> 1 year may now be salvaged with chemotherapy with or without anti-CD33 treatment ***(Chi-Kong, 2010).***

**Indication of HSCT in CML:**

Philadelphia chromosome positive CML (Ph+ CML) was previously considered as an absolute indication for HSCT including matched unrelated donor (MUD). With the introduction of imatinib and other tyrosine kinase inhibitors (TKI), adult hematologists now will not perform HSCT in CML adults and all patients will be treated with medical treatment first. Only those patients who do not respond to TKI or develop blastic phase are considered for HSCT. The situation in children is very different; the outcome of HSCT in children with CML is