Allogenic Hematopoietic Stem Cell as Curative Treatment in Myelofibrosis

Wardhana, E.A. Datau, Linda W.A. Rotty, Harlinda Haroen

Department of Internal Medicine, Siloam International Hospitals. Siloam Hospitals Group’s CEO Office, Siloam Hospital Lippo Village 5th floor Jl. Siloam no. 6, Karawaci, Indonesia.

Correspondence mail to: wadiswas@yahoo.com

ABSTRACT

Myelofibrosis (MF) is one of the Philadelphia chromosome-negative clonal myeloproliferative disorders or chronic myeloid disorders, and it is caused by much deposit of collagen substances in bone marrow, definitely is classified as hematopoietic stem cells clonal abnormality, and related to chronic myeloproliferative disorders characterized by striking figure of extra-medullary hematopoiesis.

Symptoms and signs of MF are included the variable degree of cachexia and marked extra-medullary hematopoiesis. The results of laboratory studies at presentation include anemia, leukocytosis or leucopenia, a left-ward shift in the granulocyte count, increased or decreased platelet count.

Many conventional treatment modalities have been used in the MF treatment as supportive treatments. There is only one curative treatment in MF patients using allogenic hematopoietic stem cell transplantation (HSTC). The umbilical cord blood (UCB) as the source of stem cell has increased recently and gives promising results on MF.

Key words: myelofibrosis, curative treatment, allogenic hematopoietic stem cell transplantation.

INTRODUCTION

Myelofibrosis (MF) is a disorder caused by excessive deposit of collagen substances in bone marrow, classified as a hematopoietic stem cells clonal abnormality, related to chronic myeloproliferative disorders, characterized by striking figure of extra-medullary hematopoiesis. Since this disease was reported for the first time by Heuck in 1879, and it has been known by more than 30 names, the most frequent is known by myelofibrosis with myeloid metaplasia that is usually reserved for patients with agnostic myeloid metaplasia also known as idiopathic myelofibrosis. Population-based epidemiologic studies estimate the incidence of MF at between 0.5 and 1.5 per 100,000 population. In clinical series with large numbers of patients, the median age at diagnosis was approximately 65 years. Approximately 22% of patients may be younger than 56 years, and approximately 11% younger than 46 years.

The molecular origins of the myeloproliferative disorders have been found through the discovery of a loss-of-function mutation of auto-inhibitory domain of Janus kinase family (JAK) of protein tyrosine kinases that is involved in cytokine receptor signaling, transmembrane domain of thrombopoietin receptor, and coexistence of MPLW515L or MPLW515K and MPL wild type alleles. The symptoms and signs include a variable degree of cachexia and marked splenomegaly as the result of extra-medullary hematopoiesis in the spleen. The results of laboratory studies included the anemia, leukocytosis or leucopenia, increased or decreased platelet count.
Many conventional treatment modalities have been used in the MF treatment as supportive treatments. Currently, there is only one curative treatment for MF using allogenic hematopoietic stem cell transplantation (HSTC). The umbilical cord blood (UCB) is an alternative source of HSTC and HSTC using UCB instead of BM, and it is yielding promising results.9,10

MYELOFIBROSIS

Myelofibrosis is one of the Philadelphia chromosome-negative clonal myeloproliferative disorders or chronic myeloid disorders. It is characterized by the proliferation mainly of megakaryocytic and granulocytic elements in the bone marrow, which is associated with deposition of reticulin and collagen due to a response of marrow fibroblast to signals derived from hematopoietic clone, and the circulating immature hematopoietic cells and extra-medullary hematopoiesis.2,11

The JAK2V617F mutation leads to the phosphorylation activity of JAK2, which can bind to a cytokine receptor and promote signal transducers and activators of transcription (STAT) recruitment. This mutation is likely the cause of the hypersensitivity to cytokines that characterized hematopoietic progenitor cells of MPD. Marrow cells transduced with JAK2V617F result in a clinical phenotype that closely resembles polycythemia vera, including erythrocytosis, extra-medullary hematopoiesis, and marrow fibrosis.3,4 The JAK2V617F mutation is homozygous in 13% of patients with MF, and it has been attributed to homologous recombination, which is associated with a more frequent occurrence of additional un-favourable cytogenetic abnormalities (Figure 1).12,13

There are additional somatic mutations of transmembrane domain of thrombopoietin receptor and coexistence of MPLW515L or MPLW515K and MPL wild type alleles which have been identified in MF patients and likely play a role in the biogenesis of MF. About 30% of patients with MF have a thrombopoietin protein receptor and JAK2V617F mutations, and the expression of MPLW515L results in a rapidly progressively, fully penetrable, and lethal myeloproliferative disorder, which may originate in a cell capable of generating both myeloid and lymphoid cells such as the pluripotent HSC.5 Another additional genetic event that might play a role in MF are involving 9p, 2q, 3p, chromosome 4, 12q,12q and the unbalanced translocation between chromosomes 1 and 6 with specific breakpoints (11,6).14 The differentiation program of CD34+ cells in MF, following the HSC transplantation, producing greater numbers of CD34+, CD33+, and CD41+ cells but fewer CD19+ cells, predisposing a large numbers of production of resistant to undergo apoptosis due to over-expression of the anti-apoptotic factor Bcl-xL and greater proliferation activity of MK and accumulation of MK Bcl-xL.15,16

Initial symptoms and signs of MF including variable degree of cachexia, consisted of fatigue, weight loss, sweats, and low-grade fever, which are related to tumor bulk and hypercatabolic state of increased cell turnover, and marked splenomegaly as the result of extra-medullary hematopoiesis in the spleen.6 The other sites of extra-medullary hematopoiesis including the lymph nodes, serosal surfaces leading to pleural effusion and acites, thorax especially lungs leading to pneumonia-like process, urogenital system causing hematuria, paraspinal and epidural spaces leading to compression of the spinal cord and nerve roots, liver and kidneys.7

The results of laboratory studies of MF include anemia, anisopoikilocytosis with teardrop-shaped red blood cells, leukocytosis or leucopenia, a leftward shift in the granulocyte count, increased or decreased platelet count, increased levels of lactate dehydrogenase, and increased levels of circulating CD34-positive hematopoietic progenitor cells.8 About 20% of the patients presented with a hemoglobin (Hb)
level of less than 8 g per deciliter, and in another 30% the Hb level was between 8 and 10 g/dL, and 9% of the patients had a white-cell count of more than 25,000/mm³, whereas in 7% the count was below 3,000/mm³. Extreme thrombocytosis with large abnormal platelet (≥500,000/mm³) that was observed in 13% of the patients; in 37% the platelet count was less than 150,000/mm³. The bone puncture aspiration will not succeed to be performed because of the drytap of the bone marrow and usually it needs bone biopsy to confirm the diagnosis of MF which may shows increased fibrous tissue with variable amount of megakaryocytes.  

Many conventional treatment modalities have been used in the MF treatment, as supportive treatments which are directly referred to complications of the disease. Some of the treatments that are already used in treating MF, such as thalidomide, immunomodulatory imides (IMiDs), selective cytokine inhibitory drugs, farnesyl transferase inhibitors, etanercept, imatinib mesylate, and pegylated interferon, hydroxyurea, corticosteroids, androgens, erythropoietin, anagrelide, thalidomide, pirfenidone, suramin, including repeated transfusions, spleen irradiation, and splenectomy. Anemia and thrombocytopenia may be present and are continue until symptoms developed and repeated blood transfusions are needed accompanied by the possibility of allergic reactions to the components of it, such as anaphylactic shock, due to IgE mediated against foreign protein, IgE mediated against a hapten-self protein conjugate, complement activation with anaphylatoxin generation, and direct activation of mast cells, a reaction consisting of fever, chills, myalgias, and dyspnea, with or without hypotension may be present if the antigen is leukocyte cell surface proteins, and the ABO red blood cell mismatching can result in severe hemolytic transfusion reactions causing acute renal failure, shock, and death. The treatment responses in MF with myeloid metaplasia is evaluated according to the international working group (IWG) consensus criteria.  

The removal of the spleen as a therapeutic intervention in MF has been investigated and considered, since it is related to the splenomegaly-associated complications (mechanical discomfort, refractory anemia, hypercatabolic syndrome, frequent blood transfusions, and portal hypertension). The improvement of surgical technique, patient selection, and perioperative care have made splenectomy in patients with MF recently, result in safer and more accepted practice, with reported peri-operative mortality rates 7% to 15%. Splenectomy is an additive strong independent risk factor for transformation into acute leukemia, and the blast transformation rate in splenectomized patients was 26.4% whereas it was 11.9% in non-splenectomized patients, and the cumulative actuarial transformation rate at 12 years after diagnosis was 55% in splenectomized and 27% non-splenectomized patients. The widely used risk assessment tool to assess the prognosis is the lille scoring system (Table 1). MF carries the worst prognosis among chronic myeloproliferative disorders, with median survival of 3.5 to 5.5 years, with leukemic transformation, infections, bleeding, thrombosis, heart failure, liver failure, solid tumor, respiratory failure, which include drugs, and splenectomy, as the cause of death.  

<table>
<thead>
<tr>
<th>Table 1. Risk assessment in primary myelofibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lille system</strong></td>
</tr>
<tr>
<td>Poor prognostic factors</td>
</tr>
<tr>
<td>- Hgb &lt; 10 g/dL</td>
</tr>
<tr>
<td>- WBC &lt; 4,000/µL</td>
</tr>
<tr>
<td>- WBC &gt; 30,000/µL</td>
</tr>
<tr>
<td><strong>Scoring system</strong></td>
</tr>
<tr>
<td>No. of factors</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

**ALLOGENIC HEMATOPOIETIC STEM CELL**  
Recent breakthroughs in stem cell research have encouraged the scientific community and ignite hopes for millions of patients with various disorders all around the world, including those with hematologic disorders. Human stem cells (HSC) are cells that have a greater potential to form many differentiated cell types and have potentially useful to regeneration of the tissue damaged by disease or injury. The stem cells are cells with both self-renewal capability and ability to rise to differentiated cell lines, characterized by continued ability to proliferate so that a pool of cells is always available for further use, and the ability to respond to appropriate signals by differentiating into one or more differentiated cell types.  

There are 2 types of HSC; the embryonic stem cells taken from the inner cell mass of young embryos and adult stem cells taken to mean any postnatal tissue source, including umbilical cord blood and the solid umbilical cord matrix, placental tissue, and most or all body tissues, such as hematopoietic, neural, skin, mesenchymal, and heart. The allogenic term refers
to a kind of transplantation carried out with different donor and recipient, but still in the same species, and it is the most frequent transplantation in humans.\(^{24,25}\) The hematopoietic stem cells are cells which come from the hemangyoblast differentiation which may also differentiate into endothelial cells progenitor and may be found in peripheral blood and umbilical cord, which have a capability to form all type of blood progenitor cells to maintain the hematopoiesis and the body immune function, also into somatic cells, such as: neuron, cardiomyocyte, pulmonary epithel, and other cells.\(^{25}\)

Most HSC are in the GO phase of the cell cycle, and, therefore, only a small number of stem cells are responsible for stem cell maintenance and for producing mature cells at any specific time.\(^{26,27}\) There are 3 surface markers of hematopoietic stem cells; the CD14, CD34, and CD45. The CD34 is usually used in identification and isolation of hematopoietic stem cells from blood or other tissues, and also has a role in the attachment of hematopoietic stem cells to the stromal of bone marrow.\(^{6,25}\) The CD34 is a surface glycoprotein expressed on developmentally early lymphohematopoietic stem and progenitor cells, small vessel endothelial cells, and embryonic fibroblast. Immunoaffinity-purified CD34+ marrow and cord blood cells are 10 to 100 fold enriched in colony forming units (CFUs). The CD34+ are including CFU-macrophage (CFU-M), CFU-granulocyte (CFU-G), CFU-GM, burst forming units erythroid (BFU-E), CFU-mix and CFU-blast. There are 2 distinct populations of CD34+; the CD34bright (majority of the immature hematopoietic progenitor cells) and the CD34dim (contains more lineage committed progenitors).\(^{26,28}\) The \(\text{Ceprate}\) stem cell is a tool as concentrator, based on immune-adsorption and indirect immune-magnetic beads, which may employ biotinylated 12.8 monoclonal antibody, and the sensitized cells are applied to a column of avidin-coated polyacrylamide beads, which may allow to retain the cells that expressed the CD34 antigen and unlabelled cells washed through column with gentle mechanical agitation, then the CD34+ cells are removed from the beads and collected.\(^{29}\)

The HSTC has become an increasingly used treatment modality for both malignant and non malignant disorders in the over last 40 years. About 60% of patients requiring HSTC will not have a suitable related donor, the applicability of HSTC to large numbers of patients has been augmented with the increasing availability of unrelated donors. The alternative HSC sources include unrelated donors (URD) bone marrow (BM) or peripheral blood stem cells (PBSC) and unrelated donor UCB.\(^{30}\) The UCB has been used recently and gives promising results, since it has been reported to have highly rich source of progenitor cells of all kinds and possess a naive phenotype, reduced alloreactivity, and have weak natural killer cells (NK) activity, which may reduce the frequency of graft versus host disease (GVHD) and less stringent criteria for HLA matching for donor-recipient selection.\(^{10,31}\)

**ALLOGENIC STEM CELL TREATMENT IN MYELOFIBROSIS**

Principally, there are 2 routes of distribution of the HSTC; directly implemented to the organs or tissues, and injection through the blood vessels, and after they are injected into the blood vessel, they will automatically be heading to the bone marrow.\(^{24}\) The small blood vessels in human BM, the sinusoid in which trans-endothelial migration is taking place, are composed of specialized cell structures that regulate cell trafficking. The Granulocyte-colony stimulating factor (G-CSF) may induce mobilization, increases the permeability, and creates larger gaps between BM endothelial cells. The chemokine stromal derived factor-1 (SDF-1) and its receptor (CXCR4) are expressed by immature human osteoblast in the endosteum region (Figure 2).\(^{32}\)

The SDF-1/CXCR4 expression is regulated by hypoxia inducible factor-1 (HIF-1) and since the BM is partially hypoxic, which are expressed by

![Figure 2. Stem cell (human CD34+/CXCR4+) homing to the endosteum.](image)
many cell types including immature and mature hematopoietic cells.33 The SDF-1 increased homing of human mobilized and UCB CD34+ enriched to BM. The pre-stimulation of UCB CD34+ cells with stem cell factor (SCF) induces surface CXCR4 expression and the appearance of cycling G1 CD34+/CD38+ cells with increased production of enzyme matrix metalloproteinase 9 (MMP-9) and SDF-1, which directed migration and homing to the BM and the repopulation.34 The important component of the CXCR4 signaling pathway is atypical protein kinase Cζ (PKCζ) which will be translocated into the cell membrane upon SDF-1 stimulation. The CD44 as adhesion molecule is expressed by human CD34+ cells, and its ligand hyaluronic acid (HA) expressed in BM sinusoid endothelium and endosteum region are essential for homing and repopulation, which mediate the rolling process on endothelial E/L-selectin ligand.35–37 The c-kit expression as cytokine receptor and adhesion molecule is also related to mobilization and in adhesion of hematopoietic stem cells to BM microenvironment.38

Individual cytokines are known to trigger many intracellular signal transduction pathways which presumably activate multiple gene transcription pathways to support primitive HSC proliferation or expansion. The homeobox (Hox)B4 gene is highly expressed in primitive HSC that will expand it over 100x. The protein NF-Y, a normal transcriptional activator for Hox genes, will increase HSC numbers by 20x following transplantation.39–40 The efforts at enhanced homing and engraftment of UCB HSC are including the CD26/dipeptidylpeptidase IV (DPPIV) inhibition which may enhanced the homing and engrafting capability, competitive repopulating and self renewing, fucosylation of the CD34+ UCB HSC cells which may increase the binding of these cells to P- and E-selectin expressed on endothelial cells and in the enhanced engraftment of these cells to BM, and prostaglandin E2 which may enhance engrafting capability and self renewal of UCB HSC.41

Before the HSTC transplantation is carried out, there should be a conditioning treatment in MF patients to reduce the potential of the complications, such as GVHD and rejection due to the transplantation. The conditioning treatment will directly deplete the CD4+ lymphocytes and the CD8+ lymphocytes. The introduction of reduced-intensity conditioning regimen is based on the concept of shifting the emphasis of eradication of tumor cells from high dose chemotherapy, using busulfan and cyclophosphamide or radiotherapy, into the donor cell-mediated immunologically GVHD.42 The potential advantages are low regimen-related morbidity and mortality and applicability to older patients or patients with clinically significant comorbid conditions do to toxicity of the chemotherapeutic agents used in conditioning treatment.11,43

The recently developed non-myeloablative or reduced-intensity conditioning (RIC), which are mainly based on fludarabine or low-dose total-body irradiation, which may allow stable engraftment of allogenic stem cells from related and unrelated donors in MF patients compared to the conventional or myeloablative conditioning.8,44 The splenomegaly itself may lead to sequester of donor cells after HSC, and may cause the delayed of engraftment or even graft failure. Indeed some reports have shown faster engraftment in splenectomized patients which may allow hematopoietic recovery without increased rate of the GVHD, characterized by significantly faster recovery of neutrophil and platelet counts.15,45 The UCB units should be matched at a minimum 3/6 HLA loci and should be achieving the cell dose threshold 2.5x107/kg body weight (BW). On the basis of clinical data demonstrating increasing importance of cell dose with increasing HLA mismatch. An 8/8 HLA-matched UBC HSTC remains the gold standard, and an adequate single unit of: >3.0x107 nucleated cells/kg BW for 6/6 HLA-matched units, >4.0x107 nucleated cells/kg BW for 5/6 HLA-matched units, and >5.0x107 nucleated cells/kg BW for 4/6 HLA-matched units.29 The second allogenic stem cell transplantation may be an effective salvage treatment in some patients after an initial transplant, which are conducted if there is either relapse of the disease or graft failure.46 The treatment of the prophylaxis for the possibility of post transplantation’s GVHD may include a 2 drugs combination of a calcineurin inhibitor, such as tacrolimus or cyclosporine, along with anti metabolite such as methotrexate or mycophenolate mofetil (MMF), and anti thymocyte globulin (ATG).47 The recovery of granulocytes and platelets may occur in HSC using G-CSF occurred on day 13 and 10.29

There are complications in MF patients who had UBC HSTC. The complications are including the recurrence or relapse of the disease (25%), infections (26%), acute GVHD (25%), chronic GVHD (11%), graft failure (11%), and graft rejection (4%).46 The infections usually happened in post-transplantation period, as the result from the prolonged engraftment and the immune suppression because of the use of chemotherapeutic agents to prevent GVHD.25,30 The GVHD is a major toxic effect associated with transplantation, reduces the incidence of response
and survival in UCB HSTC patients. The host’s antigen-presenting cells (APCs) are activated by the conditioning regimen, which may enhance recognition of donor T cells due to the increased expression of HLA. The T cells are activated after interaction with the host APCs, resulting in the proliferation, differentiation, and secretion of cytokines, such as IL-1 and IFN-γ. These cytokines may amplify cytotoxic T cell (CTL) and NK cell responses which, in turn, prime mononuclear phagocytes to produce TNF-α and IL-1, which may generate chemokines with mobilize effector cells to target organs. The mononuclear phagocytes are also stimulated by lipopolysaccharide (LPS) of local tissue injury that may occur via the inflammatory response. Both responses, the inflammation and increased production of CTL and NK cells, may lead to target tissue damage in transplant host.48,49

The HLA region is a multigenic system that encodes structurally homologous cell surface glycoproteins, characterized by a high degree of allelic polymorphism in humans, and the immune may give response against incompatible HLA antigens, which may act as a major barrier to HSTC. The HLA comprises 12 classical genes located on a 3.6 Mb segment of the short arm of chromosome 6. There are 3 HLA class I genes, the A, B, and C, which encode for the heavy chains of HLA-A, -B, and –C antigens. The HLA class II antigens, the DR, DQ, and DP, are heterodimers encoded by an α-chain and β-chain genes, and because of the co-dominant expression of HLA genes, a heterozygous individual may, therefore, express up to 12 different antigens.48,50 The gold standard matching comprises the analysis of HLA-A, -B, -C, -DRB1, and –DQB1 loci, and when patient and donor share the same alleles on both haplotypes the situation, refers to 10/10 match, is an estimation to allow an early decision to UCB HSTC. The risk of GVHD which may happen if recipient alleles are absent in the donor, and graft failure if donor alleles are absent in recipient, and they may increase with the number of HLA disparities and the non-shared haplotype. If there is no well matched, such as 10/10, 9/10, or possibly 8/10, from unrelated donor, or if the time frame is too short, choose a cord blood unit, as long as the number of nucleated cells is >2x10^7/kg and there are no more than 2 HLA mismatched (4/6) (Figure 3).50

In the collaborative study in European group with patients who received HSTC from HLA-matched siblings or alternative donors, resulted in the 5-year probability of survival was 46% for all patients, and the 1-year probability of treatment related mortality is 27%.51 The prospective trial of the chronic leukemia working party of the European group for blood and marrow transplantation, with MF patients at median age of 55 years with peripheral HSTC, the non-relapse mortality at 1 year was 19% and was significantly lower for patients younger than 50 years of age, and the 3-year overall survival and event free survival was 70% and 55%.52 Another study revealed the result that allogenic hematopoietic stem cell transplantation offers a 58% 3-year survival rate with a 32% relapse-free mortality rate.10

CONCLUSION

Myelofibrosis is one of the Philadelphia chromosome-negative clonal myeloproliferative disorders or chronic myeloid disorders, characterized by the proliferation mainly of megakaryocytic and granulocytic elements in the BM which is associated with deposition of connective tissue. Nowadays, the unrelated donor UCB has been chosen an alternative source of HSTC and it gives promising results, since it has a highly rich source of progenitor cells of all kinds and possesses a naïve phenotype, reduced alloreactivity, and weak NK activity. The use of UCB, as a source of stem cells, may have a role as curative treatment in MF which results in less causing GVHD and stringent criteria for HLA matching for donor-recipient selection as curative treatment of MF.

REFERENCES