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Allogeneic hematopoietic stem cell transplantation for myelofibrosis

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Primary myelofibrosis (PMF) or myelofibrosis secondary to polycythemia vera (PV-MF) or essential thrombocythemia (ET-MF) can be cured only by means of allogeneic hematopoietic stem cell transplantation (HSCT).[1] However, the age of the patient, the number of circulating blasts, cytogenetic abnormalities, the type of conditioning regimen, previous splenectomy, or selection of an unrelated donor, are among the factors that have been reported to possibly affect the outcome of HSCT.[2] Patients with disease transforming into acute leukemia have no or little benefit from transplant. On the other hand, the indication for an allogeneic HSCT in patients with no adverse prognostic factors, such as anemia or an abnormal white cell count according to the Lille scoring system,[3] but with symptoms often due to enlarged splenomegaly is still debated. More recently, it has been proposed that a low number of circulating platelets or an increased number of monocytes[4] can be added to the parameters utilized in the Lille scoring system to better stratify patients with PMF with different degrees of risk. In addition, unfavorable cytogenetic abnormalities, such as those other than 13q- and 20q-, have been associated to adverse prognosis independently of blood cell counts in patients with secondary myelofibrosis.[5]

Recent retrospective analyses of series of patients transplanted with standard myeloablative or heterogeneous reduced intensity conditioning (RIC) regimens have encouraged many centers to consider a transplant option in the management of myelofibrosis patients. Nevertheless, these studies also raised different views on whether the conditioning regimen should be based on the patient's characteristics or not.

Another controversial point that has not yet been definitively solved is what role the presence of extramedullary hematopoiesis may have in the outcome of HSCT, in particular when it causes extremely enlarged splenomegaly.

What conditioning regimen?

Allogeneic HSCT can completely reverse the fibrosis in the bone marrow,[6] restore a normal hematopoiesis and cure patients with PMF or PV-MF, or ET-MF. Retrospective studies from single institutions or co-operative groups analyzed the outcome of HSCT utilizing myeloablative conditioning regimens. A first study[7,8] demonstrated that a myeloablative HSCT, mostly utilizing total body irradiation (TBI), was effective particularly in patients with low-risk disease (85% survival rate) as compared to high risk (35% survival rate), and in patients younger than 45 years (62% survival rate) as compared to older patients (14%). The same study also showed that T-cell depletion of the graft reduces the survival of transplanted patients, suggesting a graft-versus-myelofibrosis effect from donor lymphocytes. The same effect was then supported by the successful use of donor lymphocyte infusion (DLI) in patients relapsed after HSCT.[9] Another important retrospective study[10] demonstrated a significantly better outcome in patients condi-

tioned with busulfan/cyclophosphamide as opposed to TBI/cyclophosphamide as myeloablative conditioning regimen. The possibility of transplanting myelofibrosis patients older than 45 years without unacceptable toxicities was initially demonstrated in 4 patients by using a reduced intensity conditioning (RIC) regimen with fludarabine/melphalan.[11] Larger series of patients were then reported in two other studies. The first retrospectively analyzed 21 patients,[12] all at intermediate/high risk according to the Lille scoring system and with a median age of 54 years, who were prepared with different RIC regimens and received an HSCT from matched related donors. The non-relapse mortality (NRM) at one year was 10% and the overall survival at 2.5 years was 85%. Similar good results were then reported in a prospective study[13] in 21 patients, median age 53 years, who received a conditioning regimen with fludarabine, low-dose busulfan and anti-thymocyte globulin (ATG) before receiving a matched graft from related or unrelated donors. In this study, the NRM at one year was 16% and the overall survival 84% at three years.

Differences between myeloablative or RIC regimens in published data may depend not only on the intensity, but also on the type of myeloablative or RIC regimens utilized. Patriarca *et al.*[14] now report on the 20 years (1986-2006) experience of transplantation in myelofibrosis within the Gruppo Italiano di Trapianto di Midollo Osseo (GITMO). One hundred myelofibrosis patients, median age 49 years, received a myeloablative (48%) or a RIC (52%) stem cell transplant from related (78%) or unrelated (22%) donors. Interestingly, no difference was observed in the outcome of patients receiving a myeloablative or an RIC HSCT. The relapse rate at two years was 41% and the overall survival at 34 months was 39%. Factors favorably affecting the outcome were found to be: year of transplant (after 2001), a shorter interval between diagnosis and HSCT, and the use of peripheral blood stem cells (PBSC) as compared to bone marrow cells. Although this represents the largest series of patients with comparable numbers of patients transplanted with myeloablative or RIC regimens, it is still difficult to draw a final conclusion on the role of each type of regimen due to the limitations of a retrospective study performed over many years.

While it seems reasonable to utilize an RIC regimen in elderly patients, it is still debated whether an RIC regimen should also be offered to younger patients. Current available data suggest that RIC regimens with alkylating agents (melphalan or busulfan) are very effective against myelofibrosis and cause limited transplant-related morbidity and mortality. The use of the i.v. formulation of busulfan has certainly decreased the toxicity of this compound. Moreover, it was recently shown that patients who were prepared with fludarabine and myeloablative doses of i.v. busulfan or a reduced intensity regimen with fludarabine and melphalan had comparable hematologic and extra-hematologic toxicities.[15] Since encouraging results were observed in patients receiving the combination of fludarabine and a low dose of busulfan,[13] the question of what type of conditioning regimen to use could be addressed in the future by comparing reduced *vs.* myeloablative doses of busulfan and fludarabine.

Due to the unfavorable results of HSCT in patients with acute myeloid leukemia (AML) secondary to myelofibrosis, patients with increasing number of blasts (transforming disease) in the peripheral blood should receive a myeloablative regimen in case an HSCT is attempted.

Splenomegaly, splenectomy, or JAK-2 inhibitors?

Extramedullary hematopoiesis in the spleen is a characteristic finding in PMF or PV/ET-MF patients whose quality of life can be severely impaired when splenomegaly is extensive. The role of splenomegaly in the outcome of HSCT is not completely defined. We recently examined a small series of patients with splenomegaly who received an RIC allogeneic HSCT.[16] The spleen size was monitored by measuring the longitudinal diameter by means of ultrasound or computerized tomography. Some of the patients with extensive splenomegaly (>30 cm longitudinal diameter) experienced a prolonged time to neutrophil or platelet engraftment after transplantation. Nevertheless, over 12 months all the patients had a progressive reduction of the splenomegaly, parallel to the reduction of marrow fibrosis and no rejection was observed. Therefore, although extensive splenomegaly may result in more complications secondary to a prolonged time for engraftment, it should not prevent any patient from undergoing transplant. The indication for splenectomy in symptomatic patients with an enlarged spleen is agreed by many physicians, whereas the question of whether splenectomy prior to HSCT in cases of patients with an enlarged spleen may improve the transplant outcome has not yet been proven. An initial retrospective study[17] did not show any difference in the survival of splenectomized versus non-splenectomized myelofibrosis patients after a myeloablative allogeneic HSCT. This study, however, did not consider if any patient who was a candidate for transplant did not receive it because of complications following splenectomy.[18] In fact, splenectomy was recently associated with a high risk (29%) of complications, such as bleeding, or thrombosis, or infection, and 6.6% risk of mortality in myelofibrosis patients. The relationship between splenectomy and post-transplant relapse is controversial. A higher rate of relapse was recently observed in myelofibrosis patients who underwent an RIC HSCT after splenectomy.[19] On the contrary, another study included splenectomy as a favorable prognostic factor prior to an RIC HSCT.[20]

The recent discovery of the JAK2^{V617F} mutation in approximately more than 95% PV and 50% ET and PMF patients has opened new frontiers in the knowledge of the biology of these diseases. Initial studies in myelofibrosis patients who received an allogeneic HSCT suggested that the positivity for the JAK2 mutation does not represent a prognostic factor.[21,22] However, the detection of the mutation and/or the progressive quantitative increase after transplant may indicate an initial relapse, or persistence of the disease, thus potentially leading to immunotherapeutic decisions such as withdrawal of immunosuppression or DLI.[21]

The identification of a potentially specific molecular marker of these diseases has also rapidly prompted the development of experimental targeted therapies with inhibitors of the JAK2 gene, or of the JAK family.[23] In particular, an initial phase I clinical study with a JAK inhibitor in patients with myelofibrosis showed a more than 50% reduction of the spleen in 70% of the patients within one month of treatment, significantly improving the quality of life and the performance of the patients.[24] Nevertheless, in this initial study, the use of a JAK inhibitor did not result in significant changes in blood transfusion requirement, marrow fibrosis or number of circulating blasts. Although more studies are still ongoing and definitive results are called for, the initial findings could be of great help to the transplant community. In fact, based on these initial results, patients with the JAK2 mutation and splenomegaly, and with an HLA matched donor, could be treated with a JAK inhibitor 3-4 weeks prior to starting the conditioning regimen with the aim of reducing the splenomegaly at the time of transplant and possibly improving the engraftment. Although this seems a potentially attractive hypothesis, more information on the possible effects of JAK inhibitors on the immune reconstitution and on the graft-versus-tumor effect should be obtained.

Conclusions

RIC regimens have been shown to reduce the transplant-related mortality and to improve the outcome of myelofibrosis patients undergoing an allogeneic HSCT. Current studies will allow us to better characterize factors that may affect the risk of complications or relapse, such as the role of matched unrelated *vs.* related donors, the time from diagnosis to transplant, previous splenectomy, or the presence of extramedullary hematopoiesis in the spleen, and also in other parenchyma, such as the lungs, as recently reported.[25] The question of whether to transplant a patient at low risk remains open since these patients are likely to have the least transplant-related mortality, but may also have more than ten year survival without any therapy. In particular, in the case of a relatively young patient with a matched sibling, it is probably reasonable to monitor the patient closely and as soon any clinical or laboratory change that may be related to the myelofibrosis is observed, proceed to transplant. Patients at low risk with constitutional symptoms may still have a prolonged survival without transplant, and an HSCT, especially from an unrelated donor, may represent too great a risk.

The rapid development of clinical trials with JAK inhibitors will likely give more options for the treatment of myelofibrosis. A possible scenario may also include the use of JAK inhibitors prior to transplant, as discussed above, or during the conditioning regimen.

In conclusion, the combination of fludarabine and alkylating agents at reduced or myeloablative doses may reduce the toxicity of conditioning regimens overcoming the dilemma of mini-, RIC or myeloablative HSCT in myelofibrosis. Prospective transplant studies performed in Europe and in the US will hopefully give more information on the risk factors and the biomarkers to be considered in myelofibrosis patients.

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Adoptive T-cell therapy for malignant disorders

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Adoptive cell transfer (ACT) using *ex vivo* manipulated T lymphocytes has emerged as an important advance in cancer immunotherapy, allowing for re-education and re-setting of the host immune system. Recent technological advances, particularly the development of artificial antigen presenting cells (aAPCs) for *ex vivo* stimulation and cell expansion that improve upon nature, can re-educate T lymphocytes, enhancing their potency and function. These technologies have ushered in a new generation of cell-based immunotherapeutics.

T-cell sources and flavors

The diversity of T cells for ACT is vast given that T cells may be derived from various anatomic locations, separated into different lymphocyte subsets, enriched based on phenotypic or functional characteristics such as antigen specificity, *ex vivo* activated by numerous methods, and genetically modified to change their inherent specificity, homing capacity, function, and survival *in vivo*. Ideally, T cells for ACT would possess the following properties: i) demonstrated potency and specificity against the tumor or infectious organism, ii) efficient engraftment enabling a high effector to target ratio, iii) long-term persistence *in vivo* and memory establishment, and iv) be easily obtained and efficiently manufactured.

Naive $CD4^+$ and $CD8^+$ T cells enter developmental programs after activation that ultimately result in the generation of effector memory (TEM) and long-lived central memory T cells (TCM). Understanding the mechanism underlying memory generation is accordingly critical to the development of culture systems that optimally produce populations of TEM and TCM cells *in vitro* to establish strong antitumor responses and long-lived memory for continued immune surveillance after infusion. $CD8^+$ T cells are well-established as potent effectors of anti-tumor and -viral immune responses *in vivo*; however, for the generation and/or maintenance of $CD8^+$ T-cell memory, $CD4^+$ T-cell help is required. Co-transfer of $CD4^+$ T cells can augment tumor immunity by enhancing the survival and function of transferred $CD8^+$ T cells through the secretion of cytokines such as IL-2 and the expression of CD40L, which increases antigen-presenting cell (APC) activation. Human $CD4^+$ T cells can differentiate into multiple subsets but the potential roles of these subsets in antitumor immunity are only beginning to be understood. $CD4^+$ T-helper (Th) cells were classically separated into two different subsets, Th1 and Th2, based on their pattern of activation induced cytokine production. Another subset, $CD4^+CD25^+$ regulatory/suppressor T cells (Tregs), can suppress anti-tumor immunity and were found to be associated with poor survival in human malignancies, implying that Tregs should be depleted from T-cell populations for adoptive transfer. Recently, a newly identified inflammation-associated $CD4^+$ T-cell subset (Th17) has been shown to mediate greater destruction of large tumors in mice after ACT than both Th1 and Th2 subsets. With the broad array of T cells with distinct phenotypic and functional qualities for potential use in adoptive immunotherapy, there is a need to develop novel and specific *ex vivo* culture methods for each of these T-cell subsets.

T cells for therapy: general approaches

Two broad T-cell preparatory approaches are utilized for the *ex vivo* activation and expansion of T cells for ACT therapy, namely specific and polyclonal stimulation. The former approach relies upon the isolation and activation *in vitro* of antigen-specific T cells harvested from the selected anatomic site, followed by repetitive antigen stimulation *in vitro* to preferentially expand antigen-specific T-cell clones. In the latter approach, polyclonal *ex vivo* activation of the T cells is performed using a non-specific T-cell stimulus, such as anti-CD3 antibody, with or without anti-CD28 antibody or IL-2, which preserves the polyclonal repertoire *in vitro*. When reinfused into the patient, polyclonal T cells then respond directly to antigens presented directly on