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Implementing a new conditioning regimen for allogeneic stem cell transplantation: objectives and key factors for an experimental approach

The field of hematopoietic stem cell transplantation (HSCT) has evolved rapidly from standard conditioning with cyclophosphamide (Cy), total body irradiation (TBI) and bone marrow (BM) as a source, to many diverse conditioning regimens followed by manipulated grafts.[1]

Standard conditioning and graft

A conventional transplant remains the therapy of choice for younger patients without comorbidities in the absence of results from prospective, controlled trials.[2] A conventional conditioning is defined as a package of full myeloablation based on high doses of Cy-TBI or Cy-busulfan. Such preparative regimen is followed by infusion of marrow HSC or G-CSF-mobilised peripheral blood progenitor cells (PBSC) from an HLA-matched related or unrelated donor. For allogeneic HSCT, both sources are used as a standard, although both methods have their specific peculiarities. Peripheral blood stem cells are associated with more rapid engraftment in the recipient and an increased incidence of chronic GVHD, compared to BMT. However, unfractionated allogeneic HSCT following high-dose chemoradiotherapy is associated with a considerable risk of acute graft-versus-host disease (GVHD), leading to significant morbidity and mortality. Age is an important prognostic factor for treatment-related mortality (TRM) which increases by each decade both in HLA-identical sibling transplants and more so for alternative donor transplants.

Reduced-intensity conditioning regimens

The first historical challenge to standard myeloblative conditioning has been run during late years '90, when low-dose TBI and fludarabine containing regimens have been explored in preparation for allogeneic HSCT followed by manipulated grafts.[1] These pivotal experiences lead to confirmation that hematopoietic stem cell engraftment can be obtained with less-intensive chemo-radiotherapy, with antibodies, with low-dose radiation, with large number of stem cells. In the original meaning, donor stem cell engraftment was intended as a self-sufficient platform to cure tumor by the in

vivo development of Graft versus Tumor (GvT) effect eventually enhanced by the infusion of donor-derived lymphocytes (DLI). However, the extended experience with RIC-HSCT has demonstrated that HSC engraftment is not sufficient to cure leukemia, as shown by increased risk of relapse with the original leukemia as compared to standard myeloablative conditioning (MAC).[3] RIC transplants are therefore discouraged in patients with progressive or refractory disease.

Allogeneic transplants with reduced intensity conditioning are increasingly used for the treatment of malignant and nonmalignant diseases. During the last years, approximately 25% of allogeneic HSCT were performed with reduced conditioning regimens.[2] A wide variety of reduced intensity conditioning regimens have been described in publications and there is no general agreement on the RIC exact definition. Extensive feasibility studies have been published and short-term results clearly show that RIC HSCT can decrease the risk for early transplant-related mortality, thereby making transplants for older patients and for patients with co-morbidities possible. Consequently, the major intrinsic limitation of performing a prospective comparative trial between RIC and MAC has been the different population eligible to those treatment modalities. The need to significantly reduce the volume of the tumor with appropriate form of chemoradiotherapy, has prompted the identification of disease-specific RIC regimens.

Objectives and key factors in the design of new conditioning regimens

RIC-HSCT has reached solid evidences in terms of feasibility for all diseases candidate to allogeneic transplantation, and increase of the upper age and acceptable comorbidities limit by reducing TRM. Acute GvHD and chronic GvHD remain an issue post RIC-HSCT.

Several relevant questions can be listed as potential objectives and key factors for the development of new conditioning regimens.

New immunosuppressive agents

At present, although fludarabine has been incorporated in most RIC regimes for its marked immunosuppressive properties, no formal comparison of fludarabine with standard doses of cyclophosphamide has been provided. A prospective randomized study promoted by Rambaldi is starting within The Gruppo Italiano Trapianto di Midollo Osseo (GITMO), comparing intravenous busulfan (I.V. Bu; Busilvex®) plus fludarabine (BuFlu) versus intravenous busulfan plus cyclophosphamide (BuCy2) as conditioning regimens prior to allogeneic HSCT in patients aged ≥ 40 and ≤ 55 years with Acute Myeloid Leukemia (AML) in Complete Remission (CR).

The substitution of fludarabine with clofarabine, a new purine analogue with both immunosuppressive and antileukemic properties, in association with an alkylating agent, is an attractive option for patients undergoing HSCT with advanced leukemia.

Alkylating agents

The introduction of intravenous formulation of busulfan, has provided the tool for a wider use of this alkylating agent in centers not performing targeting drug levels during treatment. However, comparative trials using intravenous Busulfan as standard arm are still ongoing, when exploring the combination of fludarabine with others myeloablative antileukemic agents.[4]

Treosulfan is a bifunctional alkylating agent approved for the therapy of advanced ovarian

carcinoma.[5] Latest data from animal studies show that a fractionated dose of treosulfan (14 g/m^2 on day -4, -3, -2) can induce a stem-cell toxicity comparable to that of busulfan. Since treosulfan has a cytotoxic effect on a very high percentage of both the primitive and committed stem cells, allogeneic SCT can be successfully performed without the need to administer cyclophosphamide in addition. Recent results from clinical phase II studies, revealed low non-haematological toxicity in spite of a full myeloablative dose; serious liver toxicities such as veno-occlusive disease (VOD), nephrotoxicities, neurotoxicities or lung toxicities like those known to occur with other high-dose alkylating agents, were not observed in the high-dose treosulfan studies. Treosulfan-fludarabine based conditioning reports document a reduced toxicity, well tolerable and efficient regimen even in patients with high risk disease and otherwise not eligible for allogeneic transplantation. A comparative trial between treosulfan-based and i.v. busulfan based regimens is warranted.

Factors not directly related to conditioning drugs

The allogeneic HSCT consists of three components: the *preparative* conditioning regimen, the allogeneic stem-cell-graft, and the immunosuppressive prophylactic treatment for GvHD. The conditioning regimen is aimed at the suppression of the recipient's hematopoiesis and immune system and it's self-renewal capacity for acceptance of the stem-cell graft. In addition, it helps to eradicate the malignant clone and therefore the choice of conditioning regimen agents is supposed to be influenced by the disease specificity. The choice of graft source of allogeneic stem cells and the immunosuppressive treatment for GvHD-prophylaxis might significantly influence the probability of a given conditioning to provide a stable HSC engraftment and GvT effect. When designing a new conditioning regimen, both graft source and GvHD prophylaxis should be targeted to specific conditions in which the risks of acute and chronic GvHD is properly balanced to the risk of disease recurrence.

Regulatory issues

The most appropriate and reliable methodology to provide a clinical evidence to a relevant answer, is the development of a prospective clinical trial. However, the EU Clinical Trials Directive has recently given a hard time for the feasibility of Investigator Initiated Academic Clinical Trials.[6,7] The Directive was primarily developed by EU-DG Enterprise for company-sponsored studies and not for Investigator Initiated Clinical trials, thus discouraging Investigators from running clinical trials in reasons of the lack of resources requested to accomplish with the Directive reqirements. The European Blood and Marrow Transplantation Group (EBMT) is actively discussing problems arising from the Directive in Brussels with DG Sanco and DG Research, and also in London with the EMEA. One of the projects funded by the EU dealing with an analysis of problems arising from the Directive is CLINT, aimed at supporting the EBMT to develop its infrastructure for the conduct of trans-European clinical trials in accordance with the EU Clinical Trials Directive, and to facilitate International prospective clinical trials in stem cell transplantation (*http://www.ebmt.org/enew/july 2008/ebmt-newsletterh.html*).

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