

Minitransplants: allogeneic stem cell transplantation with reduced toxicity

Running title : Minitransplants

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (HSCT) is used for the treatment of selected hematological malignancies. Its curative potential is based on two very different mechanisms, involving the conditioning regimen and the graft-versus-host reactions, respectively. The high-dose chemo-radiotherapy conditioning regimen is aimed at destroying tumor cells, ablating the host immune system (to prevent rejection) and eliminating the host bone marrow (to “make space” for donor stem cells). However, the definitive eradication of tumor cells is also largely mediated by an immune-mediated destruction of malignant cells by donor lymphocytes termed graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect. However, because of its toxicity, conventional allogeneic HSCT is restricted to younger (< 55 years) and fitter patients. These observations led several groups to set up new (less toxic) transplant protocols based on a two step approach : first the use of immunosuppressive (but nonmyeloablative) conditioning regimens providing sufficient immunosuppression to achieve engraftment of allogeneic hematopoietic stem cells and, in a second step, destruction of malignant cells by the GVL effect. These transplants are called nonmyeloablative HSCT or reduced-conditioning HSCT or minitransplants. Preliminary results show that minitransplants are feasible with a relatively low transplant-related mortality (TRM) even in patients up to 70 years. In addition, strong anti-tumor responses are observed in several hematological malignancies as well as in some patients with renal cell carcinoma. As the benefits of minitransplants over alternative forms of treatment remain to be demonstrated, this strategy should be restricted to patients included in clinical trials.

INTRODUCTION

The curative potential of allogeneic hematopoietic stem cell transplantation (HSCT) is mediated not only by the eradication of malignant cells by high-dose chemotherapy (and total body irradiation), but also by an immune-mediated graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect (1,2). The power of the GVL effect and its apparent mediation by donor lymphocytes led several groups to infuse donor lymphocytes (DLI) in patients with relapsed leukemia after HSCT (3,4). The induction of durable remissions by DLI demonstrated that the GVL effect is capable of eradicating hematological malignancies even in the absence of chemotherapy. This prompted the introduction of new protocols based on the development of a GVL reaction after low-dose (less toxic) nonmyeloablative preparative regimens providing sufficient immunosuppression to achieve engraftment of allogeneic hematopoietic stem cells (figure 1).

THE GVL EFFECT

The existence of a GVL effect in humans was first demonstrated by the Seattle's group that evidenced a reduced relapse rate in patients with acute (1) and/or chronic (2) graft-versus-host disease (GVHD). This was confirmed by others groups that observed an increased risk of relapse after T cell-depleted (TCD) allogeneic HSCT as well as after syngeneic HSCT (5). The GVL effect was also demonstrated by the evolution of minimal residual disease post-transplant, which often ceases to be detectable only 6 to 12 months after HSCT and by the occurrence of GVL activity with or without GVHD after cessation of GVHD prophylaxis for post-transplant relapse (6).

This led several groups to infuse donor lymphocytes (DLI) in patients with relapsed leukemia after HSCT (3,4). DLI induce a complete remission in about 65 % of the cases in chronic myelogenous leukemia (CML) and in 20 to 30 % of the cases in acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) (3,4). Some patients with acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hodgkin's disease (HD),

non-Hodgkin's lymphoma (NHL) as well as multiple myeloma (MM) have also responded to DLI or discontinuation of immunosuppressive therapy (3,4). A GVT effect has also been demonstrated in breast cancer (7) and in renal cell carcinoma (RCC) (8), and possibly in ovarian (9) and non-small cell lung (10) carcinomas.

However, the GVL effect mediated by DLI needs time : the median time to achieve a cytogenetic remission was 85 (range 28 to 241) days for patients with CML (the time to achieve molecular remission can be prolonged) and 34 (range 16 to 99) days for patients with AML (11). Complications of DLI include acute and chronic GVHD and transient marrow aplasia. It is possible to reduce the risk of GVHD without impairing the GVL effect by CD8 depletion of DLI (12) or by starting with a low dose of T cells and increasing the dose in a stepwise fashion in case of no response (13).

ROLES OF THE CONDITIONING REGIMEN

Allogeneic HSCT was first considered to deliver supralethal doses of chemotherapy and total body irradiation to tumor cells. The beneficial effect of high doses of chemo-radiotherapy is illustrated by the decreased incidence of disease relapse after autologous HSCT compared to conventional therapy alone in several hematologic malignancies (14,15) (figure 2). However, a large part of the efficacy of allogeneic HSCT is mediated by immune-based GVL or GVT effects (figure 2).

Immature progenitor cells occupy defined niches within the marrow stroma in order to obtain the necessary support for proliferation and differentiation. To allow access for donor cells to these niches, it was commonly believed that host stem cells must be eradicated by the conditioning regimen. However, Storb et al recently demonstrated that the graft itself, most likely through subclinical GVH reactions, is capable to create these marrow spaces in the absence of both chemotherapy and bone marrow irradiation (16).

It is necessary to abolish host defense prior to transplantation to avoid immune-mediated graft rejection caused by alloreactive cytotoxic host lymphocytes or by HLA-specific antibodies. It was commonly believed that the conditioning regimen was critical for ensuring engraftment. However, donor T lymphocytes (and particularly donor CD8 lymphocytes) are also strongly implicated in the destruction of the host immune system (17). Therefore, TCD of the graft as a method to prevent GVHD may have deleterious effects on engraftment (18). Recently, the Seattle's group demonstrated that optimizing postgrafting immunosuppression can also control the host-versus-graft reaction (19). Thus, contrarily to TCD of the graft that prevents GVHD but increases the risk of graft rejection, optimal postgrafting immunosuppression reduces the incidence of both GVHD and rejection.

NONMYELOABLATIVE CONDITIONING REGIMENS

Because of its toxicity, conventional allogeneic HSCT is restricted to younger patients (<55 years for allograft procedures with HLA-identical siblings and < 50 years for unrelated donor transplants) without significant organ impairment. Unfortunately, the majority of malignancies potentially cured by allogeneic HSCT and for which a GVL effect has been demonstrated are more frequent in older patients. Thus, it may be important to develop less toxic approaches to allografting that can also be extended to older patients or patients with pre-existing organ impairment (figure 1).

In 1997, Giralt et al. (20) reported the engraftment of HLA-identical allogeneic HSC after nonmyeloablative chemotherapy based on purine analogs. The rationale for using purine analogs (fludarabine or 2-CDA) was their capacity to inhibit the mixed lymphocyte reaction in vitro and to produce lymphopenia and substantial immunosuppression in vivo. Other pilot trials by the same group confirmed these preliminary results and achieved durable engraftment and remissions in some patients with myeloid as well as lymphoid malignancies, with a relatively low TRM (21,22). The Jerusalem's group developed another nonmyeloablative purine analog-based protocol combining fludarabine, ATG and low-dose oral busulfan (23). This allowed

achieving engraftment and full donor chimerism in the majority of the patients with a low TRM. However, it should be emphasized that many patients included in this study would be considered eligible for conventional allogeneic HSCT. The feasibility of fludarabine-based nonmyeloablative transplant protocols has also been confirmed more recently by several others groups (24-26).

In an elegant canine allogeneic transplant model, the Seattle's group demonstrated that stable mixed chimerism could be achieved using pre-transplant low-dose TBI combined with post-grafting immunosuppression with a combination of Cyclosporine A (CsA) and mycophenolate mofetyl (MMF) and that post-grafting immunosuppression can serve to control both HVG and GVH reactions (19). Complete chimerism was achieved through DLI. Initial experience in humans showed the feasibility and safety of this approach (27). Moreover, major disease responses were observed in more than 70% of the patients who had measurable disease pretransplant and achieved sustained engraftment (27).

Finally, the Boston's group demonstrated in a murine model (28) and then in humans that mixed chimerism could be induced in HLA-matched (29) or 2 or 3 loci-mismatched (30) allogeneic HSCT by a nonmyeloablative conditioning regimen combining cyclophosphamide, thymic irradiation and ATG.

MIXED HEMATOPOIETIC CHIMERISM

Minitransplants usually result initially in mixed hematopoietic chimerism (MC) that can be defined as the presence of 1-95% hematopoietic cells of donor origin (figure 1). This state is characterized by mutual donor-host tolerance (and thus control of both GVH and host-versus-graft (HVG) reactions) while immune responses against other antigens remain normal. The mechanisms involved include central thymic deletion of both donor- and host-reactive T-cells (because both donor and host dendritic cells are present in the thymus of mixed chimera) and peripheral tolerance due to suppressor T-cells (31).

For the treatment of hematologic malignancies, mixed donor chimerism is not expected to be always curative. It is now well demonstrated that MC is associated with relapse in patients with diseases such as CML. For those patients with hematologic malignancies, MC can thus be converted to full donor chimerism (FC) by DLI (figure 1).

The assessment of hematopoietic chimerism requires more sensitive techniques than conventional cytogenetic analyses because of the availability of only small numbers of dividing cells. The most current techniques are fluorescent in situ hybridization (FISH) with X- and Y-specific probes in case of sex-mismatched transplant and polymerase chain reaction-based assays of polymorphic mini- or micro-satellite markers in case of sex-matched transplant. Other techniques based on restriction fragment length polymorphism (RFLP) are also used.

The evolution of myeloid and lymphoid chimerism after non-myeloablative HSCT may be discordant. Achievement of full donor T-cell chimerism is associated with disease regression (24). Moreover, the Seattle's group recently showed that the level of T-cell chimerism on day 28 predicted for both graft failure and acute GVHD (27), underlying the importance of lineage-specific chimerism analysis.

ENGRAFTMENT AND TOXICITY AFTER MINITRANSPLANTS

The engraftment rate is related to the intensity of the conditioning as well as the type of transplant. Generally, more intensive conditioning regimens resulted in higher engraftment rates : graft failure rates ranged from 0% to 20% of the cases in the Jerusalem's and in the Seattle's studies, respectively (23,27). Moreover, the immune status of the recipient also appeared to be important for engraftment. For example, a high incidence of graft rejection was observed by the Seattle's group in previously untreated CML patients, inducing them to add fludarabine in their "TBI only" protocol for such patients.

Generally, the conditioning regimens used in the setting of minitransplants are well tolerated, inducing little or no grade 3-4 toxicity, even in patients older than 65 years or with concomitant comorbidities. However, there are important discrepancies among the different

studies, due to the relative intensity of the regimen used, the age of the patients as well as the type of transplant (sibling versus unrelated, HLA-identical versus mismatch). The 200-day transplant-related mortality (TRM) varied from 4% in the Seattle study (27) (using low dose TBI alone as conditioning regimen in HLA-identical sibling transplants) to 37% in the Houston's study (22) (using melphalan and purine analog-containing preparative regimens in related or unrelated graft recipients ineligible for conventional transplants). Age and disease status at transplantation remain important prognostic factors for TRM.

In both animal and human studies, the use of less severe conditioning as well as the initial presence of host hematopoietic cells decrease the severity of acute GVHD. These observations predict that acute GVHD may be limited after minitransplants because of the low intensity of the conditioning and the high incidence of mixed chimerism achieved. Indeed, preliminary data suggest that acute GVHD is relatively mild and generally controllable after minitransplants (27). Moreover, acute GVHD is usually delayed and occurs after patients have recovered from conditioning-related toxicities (24,27). However, there are relatively large discrepancies among the different studies. This variability probably relates to differences in the source of stem cells (bone marrow versus PBSC), type of transplant (related versus unrelated), GVHD prophylaxis, use of ATG as well as age of the patient. In fact, acute GVHD is still the leading cause of non-relapse mortality. Additional DLI are significantly associated with increased risks of acute GVHD (29). However, the time of infusion as well as the dose of lymphocytes given play a major role.

Because of the short follow-up, the incidence and severity of chronic GVHD are still uncertain. However, preliminary trials reported the occurrence of severe chronic GVHD in some cases (21). Moreover, despite such short follow-up, the risk of chronic GVHD was already 74% in the Seattle's study (27) and 68% in the Houston's report (22).

ANTITUMOR EFFICACY

Although data are too early to definitively assess antitumor effects, preliminary results clearly demonstrate the occurrence of major disease responses in patients with hematological malignancies as well as some solid tumors.

CLL and lymphoma

Durable complete responses were observed in several patients with refractory non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD) or CLL (21,30). The Boston's group reported the evolution of 16 patients treated with minitransplant after a conditioning regimen combining cyclophosphamide, ATG and thymic irradiation for primary refractory or relapsing resistant NHL, HD or CLL. Complete responses were observed in 7/16 patients (4/11 patients with NHL, 2/3 patients with HD and 1/2 patients with CLL). Similarly, the Jerusalem's group reported on a group of 23 heavily treated high-risk malignant lymphomas (32). There were 12 patients with resistant disease and 11 with partial response, with 5 having failed a previous autologous transplant. Ten of the 23 patients were alive in CR 15 to 37 months after the transplant and the 3-year probability of disease-free survival was 40%. Kottaridis et al. reported on 14 patients with HD or NHL in partial remission (n=8) or with refractory disease (n=6) (24). The conditioning regimen consisted in fludarabine, melphalan and CAMPATH-1H. Five out of the 14 patients experienced a complete response and stabilization occurred in 7 others patients.

CML

Complete cytogenetic or molecular remissions were obtained in more than 75% of CML patients transplanted in chronic phase (22,24,25,27,33). Moreover, some patients with more advanced phase also achieved molecular remission (27).

Multiple myeloma

Durable (> 1 year) partial and complete responses were also observed in some patients with multiple myeloma (25,27,34). Badros et al. (34) studied 16 relapsing multiple myeloma patients (10 in refractory relapse, 4 with partial response and 2 with near CR) receiving a minitransplant after conditioning with melphalan 100 mg/m². After a median follow-up of 1 year, 5 patients achieved and sustained CR, 3 near CR and 4 partial response (PR). Two patients died of progressive disease and 3 died of GVHD without active disease.

AML, ALL and MDS

Storb recently reported the results of 17 AML patients treated with related minitransplant after conditioning with 2 Gy TBI ± fludarabine (90 mg/m²) (35). Eight of 10 patients grafted in CR remained in CR after 5-18 months. Moreover, 2/3 patients with primary refractory disease were in remission at more than 20 months. Prolonged remissions in refractory AML patients were also reported by other groups (22,33).

Solid tumors

In patients with solid tumors, responses were partial and transient in patients with breast cancer or melanoma, whereas some patients with RCC achieved durable complete responses (8,24). Childs et al. recently reported the evolution of 19 patients treated with minitransplant after conditioning with fludarabine and cyclophosphamide for metastatic RCC (8). Ten of the 19 patients enjoyed major responses, including 3 patients with sustained (> 20 months) complete response. These responses occurred 3-6 months after the transplant and usually after cyclosporine discontinuation. Acute GVHD was associated with disease response but, interestingly, one patient had a complete response in the absence of acute GVHD.

MINITRANSPLANT AFTER A PREVIOUS CONVENTIONAL TRANSPLANT

Treatment options for patients who relapse or develop secondary malignancies after autologous or allogeneic HCT are limited. In these patients, results of a second allogeneic HSCT are generally poor, primarily because of a high rate of TRM. Recently, the Jerusalem's group studied the feasibility of a second allogeneic HSCT after a nonmyeloablative conditioning regimen (36). Among the 12 patients included, only one died of procedure-related complications, suggesting that low-intensity regimens significantly reduce TRM associated with second transplants. Moreover, the actuarial disease-free survival at 34 months was 50%. These findings were confirmed by Kottaridis et al. who reported a 14% TRM associated with an allogeneic minitransplant for disease relapses occurring after standard autologous or allogeneic HSCT (25). We also reported a low incidence of transplant-related mortality in patients receiving a minitransplant after relapsing after a conventional autologous transplantation (37).

For patients with high tumor burden, the Genoa's group studied the feasibility of conventional autologous HSCT followed by a minitransplant 1 to 3 months later (26) (figure 2). The rationale for high-dose therapy followed by autologous HSCT was debulking and the rationale for minitransplant was to induce immune-mediated anti-tumor effects. The rationale for separating high-dose therapy from allogeneic transplantation was to reduce the TRM and the risk of acute GVHD (see above). Preliminary results evidenced the feasibility of this approach with a low TRM (26).

T CELL DEPLETION OF THE GRAFT

It is now well demonstrated that a conditioning regimen-related cytokine storm plays a major role in the pathogenesis of GVHD. Moreover, in the minitransplant setting, it is well demonstrated that donor lymphocytes given several weeks after the transplant in mixed chimera induce significantly less GVHD than a similar dose of donor T-cells given together with the transplant, without reducing their anti-tumor efficacy (28). We have recently reported that transplantation of CD34-selected allogeneic PBSC after a myeloablative preparative regimen

followed by pre-emptive CD8-depleted DLI significantly decreases the incidence of acute and severe chronic GVHD as compared with unmanipulated BMT (38). We also investigated the feasibility and efficacy of minitransplants with CD8-depleted or CD34-selected PBSC followed by pre-emptive CD8-depleted DLI given in incremental doses on days 40 and 80 (depleted group). None of the ten patients included in the depleted group versus 3/4 recipients of unmanipulated PBSC and DLI experienced grade II-IV acute GVHD. Most of the patients included in the depleted group were mixed chimera on day 30 but became full donor chimera after CD8-depleted DLI (37).

Kottaridis et al. (25) recently investigated a novel nonmyeloablative conditioning regimen consisting in CAMPATH-1H, fludarabine (150 mg/m²) and melphalan (140 mg/m²). They observed a high engraftment rate (> 97%) but most of the patients analyzed were mixed chimera. The incidence of GVHD was exceptionally low (5% of grade II-IV acute GVHD). The authors explain this observation by the use of in-vivo CAMPATH-1H (achieving in vivo T-cell depletion of the graft because of its prolonged half-life in humans) and by the high incidence of mixed chimerism (known to reduce the incidence and severity of GVHD). However, as mixed chimerism may diminish the GVL effect seen in the allograft setting, longer follow-up is needed to clarify if this approach respects the GVL effect.

CONCLUSION

In conclusion, minitransplants are feasible and can lead to molecular responses. This transplant strategy offers several advantages over conventional HSCT: [1] TRM is reduced; [2] acute GVHD could be less frequent and less severe than after myeloablative HSCT; [3] minitransplant are possible in patients older than 55 or with concomitant comorbidities. Further clinical trials are needed to define more effective strategies to separate GVL effects from GVHD and to compare the relative efficacy of this approach to conventional treatment (39).

The potential indications for a minitransplant include the same disease indications as for a standard transplant but in patients unfit for a myeloablative conditioning regimen because of age

(> 50-55 to 70 yrs) or poor clinical condition (infection, organ failure). It is not unlikely that minitransplants could replace standard transplants even in young fitter patients for diseases such as CML. In addition, minitransplants may be used in diseases where GVT effects are more important than high-dose chemotherapy, such as renal cell carcinoma. In the future, the potential of minitransplants to replace a diseased hematopoietic or immune system may also be exploited to cure non-malignant hematological disorders (such as aplastic anemia, thalassemia, sickle cell disease or SCID) or autoimmune diseases (such as rheumatoid arthritis, multiple sclerosis or sclerodermia). Finally, combination of HSC and solid organ transplants from the same donor may provide definitive tolerance toward the transplanted organ and eliminate the need for prolonged immunosuppression.

As the benefits of minitransplants over alternative forms of treatment remain to be demonstrated, this strategy should be restricted to patients included in clinical trials.

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LEGEND TO THE FIGURES

Figure 1 : Schedule of minitransplant. Pre-transplant recipient immunosuppression is carried out with either low-dose chemotherapy or chemo-radiotherapy. Patients receive donor HSC on day 0. Post-grafting immunosuppression is carried out with cyclosporine A with or without MMF or MTX. DLI are given 30-100 days after the transplant in case of mixed chimerism and/or residual disease to obtain full donor chimerism as well as eradication of tumor cells.

○ Cell of host origin. ● Cell of donor origin. ☼ Host tumor cell.

Figure 2 : Comparison of various approaches to allogeneic HSCT.

- A. Conventional allogeneic transplantation. After a complete remission (CR) is achieved through standard chemotherapy, conditioning with massive doses of chemo-radiotherapy further reduces residual disease that is finally eradicated by the GVL effect.
- B. Minitransplant. After low-intensity conditioning that has little impact on the tumor, transplantation of allogeneic stem cells and further DLI are responsible for the eradication of the tumor.
- C. Conventional autologous transplantation followed by a mini-allotransplant. After initial CR, high-dose conditioning (with autologous HSCT rescue) further reduces residual disease. Then, a minitransplant is carried out. This is the equivalent of performing a standard allogeneic transplant in 2 steps, i.e. a first step of chemotherapy intensification (autologous transplant) and a second step of adoptive immunotherapy (minitransplant).

Figure 1

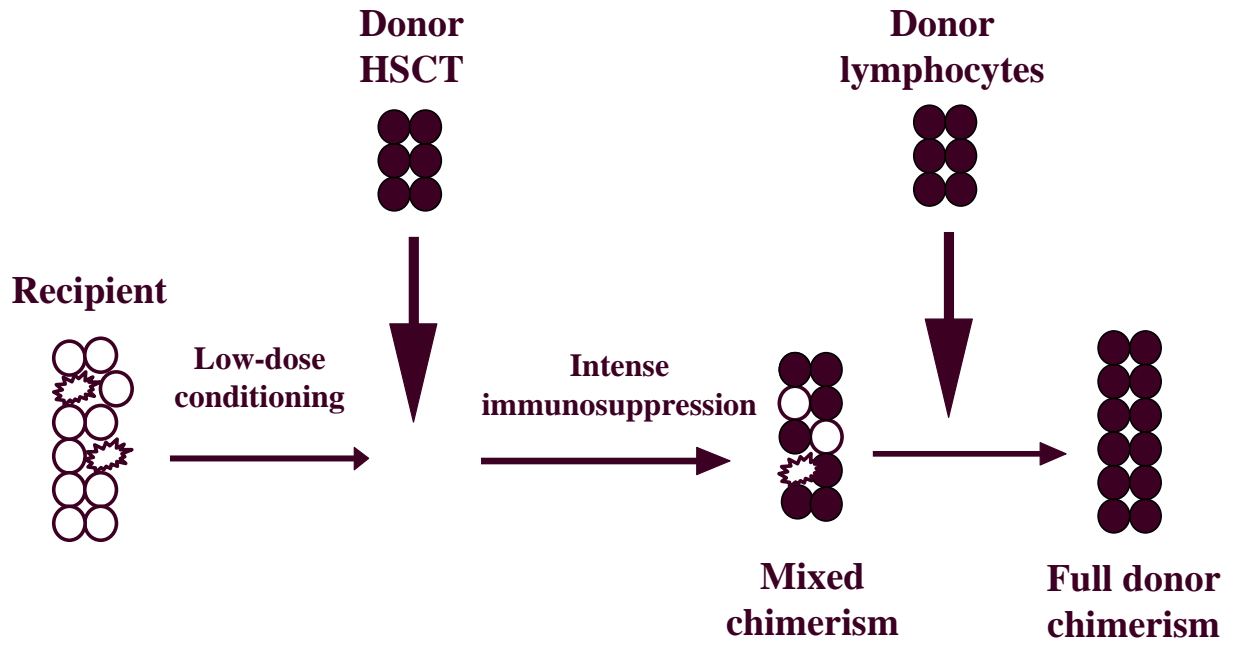


Figure 2

