Cell- and drug-based prevention of relapse after allogeneic hematopoietic stem cell transplantation

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A B S T R A C T

Over the past years a remarkable improvement in the clinical outcome of allogeneic transplantation (alloHSCT) has been achieved, mainly through a reduction of non-relapse mortality. This is particularly relevant when considering that alloHSCT is now offered to patients of an age that may extend up to the seventh and eighth decade. Relapse of the primary neoplastic disease remains an open issue requiring extensive laboratory and clinical investigation. Innovative post engraftment treatments should now be regarded as an integral part of the alloHSCT platform, and clinical trials appropriately designed to address efficacy and toxicity of such new approaches are needed.

Learning goals

At the conclusion of this activity, participants should have been updated about:
- current integrated platforms with post-conditioning drugs and unselected donor lymphocytes;
- innovative post-transplant target therapies with *in vitro* selected cells or new drugs.

Introduction

Disease relapse after allogeneic transplantation (alloHSCT) largely remains an unmet clinical need when considering that approximately 25%-50% of transplanted patients eventually relapse. The rate of disease recurrence may exceed 30% in acute myelogenous leukemia (AML) and 40% in acute lymphoblastic leukemia (ALL). In multiple myeloma (MM), Hodgkin disease (HD) and diffuse large B-cell non-Hodgkin lymphoma (DLBCL) the rate of disease recurrence may be even higher due to the very advanced phase of the disease when the allogeneic transplant is usually offered. The probability of disease recurrence after transplant may also be remarkably different according to the age and the clinical status of patients, the type of donor, and the stem cell source, as well as the intensity of the conditioning regimens. Indeed, the development of reduced intensity conditioning (RIC) regimens and the availability of alternative donors and stem cell sources have greatly facilitated the possibility of offering an alloHSCT to many patients for whom a decreased risk of non-relapse mortality (NRM) has been documented in recent years.[1] Unfortunately, the risk of disease relapse has not been reduced significantly and remains the most common reason for treatment failure. The weakness of a reduced anti-neoplastic activity of the preparative regimen may be further increased by the use of aggressive *in vivo* T-cell depletion strategies based on the use of polyclonal or monoclonal antibodies that may cancel the potent anti-neoplastic effect of donor T lymphocytes. In fact, relapse occurs in 30%-50% of recipients of T-cell depleted alloHSCT for AML and myelodysplastic syndromes (MDS).[2]

All in all, disease relapse after alloHSCT derives from residual disease that survives the preceding chemo-radiotherapy, as well as the graft-*versus*-malignancy effect. Therefore, precise application of pre-emptive strategies when the burden of disease is minimal, and before overt relapse, is likely the most feasible and effective means. New technologies, such as molecular genetics, fluorescence *in situ* hybridization (FISH), flow cytometry and chimerism analysis, allow a more accurate prediction of disease relapse after alloHSCT, and new promising post-transplantation strategies using tumor-specific agents with the potential to reduce disease recurrence are emerging. For these reasons, and given the perception that relapse is the main problem affecting survival after transplantation, in 2009 the National Cancer Institute launched an international scientific effort to study the biology, prevention, and treatment of relapse after alloHSCT.[3] Here, we will briefly review the most recent approach to prevent disease relapse after allogeneic transplantation using cell- and drug-based therapeutic strategies (Table 1).

Prophylactic donor lymphocyte infusions

Non-Hodgkin lymphoma and acute myeloid leukemia

Prophylactic donor lymphocyte infusions (DLI) have been used to prevent disease relapse in patients with low-grade non-Hodgkin lymphoma (NHL), particularly after RIC transplants. In 82 consecutive patients with follicular NHL who underwent transplan-

tation using fludarabine, melphalan and alemtuzumab, prophylactic DLI were given to correct mixed donor chimerism from six months after transplant. By this approach, transplantations using HLA-matched or mismatched related or unrelated donors was found tolerable, with modest graft-*versus*-host disease (GvHD) rates and NRM.[4] Promising results have also been reported in patients with refractory or relapsed AML using a sequential treatment approach. After an aplasia-inducing chemotherapy consisting of fludarabine, cytarabine, and amsacrine (FLAMSA) and three days of rest, a RIC program based on 4 Gy total-body irradiation (TBI), antithymocyte globulin, and 80-120 mg/kg cyclophosphamide was given as conditioning to alloHSCT. Patients received early prophylactic DLI if they were in complete remission (CR) without evidence of GvHD at day +120 after transplantation or after discontinuation of immunosuppression. The DLI dose ranged between 1 to $5x10^6$ CD3+ cells/kg in patients without a history of acute GvHD and could be repeated up to three times at 4- to 6-week intervals. In a cohort of high-risk AML patients, these authors reported a 2-year overall survival (OS) and leukemia-free survival of 40% and of 37%, respectively.[5] A subsequent phase II study in AML patients with high-risk cytogenetic or treatment refractory disease confirmed the interesting results showing a 4-year survival after alloHSCT of 61%.[6] Similar results have been obtained using busulfan (BU) instead of TBI, always after FLAMSA.[7] So far, it is difficult to speculate if these encouraging results are mainly to be explained by the intensive course of chemotherapy, aimed at reducing leukemic burden, or by the reinforcement of the graft-*versus*-leukemia (GvL) induced by DLI. In this regard, future studies are needed to better define the optimal balance between the 2 mechanisms of anti-leukemic activity and to obtain a greater efficacy while reducing the detrimental toxic effects of chemotherapy and lymphocytes. However, the similar experience conducted in childhood AML confirming that pre-emptive immunotherapy may improve the outcome in high-risk patients after transplantation seems to suggest that the main role of this combined approach is played by the adoptive immunotherapy. Indeed, Rettinger *et al*. showed that treatment with DLI of patients with mixed chimerism was able to restore a complete donor chimerism in approximately 50% of patients without toxicity, and these patients remained in long-term remission, while all patients failing to achieve such a response relapsed.[8]

With or without tyrosine kinase inhibitors in chronic myelogenous leukemia and Philadelphia positive acute lymphoblastic leukemia

Since the introduction of several tyrosine kinase inhibitors (TKIs), the therapeutic landscape of chronic myelogenous leukemia (CML) has dramatically changed[9-11] and alloHSCT is no longer the first choice approach as it was during the 1990s, even for young patients in first chronic phase. Despite the impressive therapeutic progress achieved by the use of TKIs, alloHSCT remains a curative treatment option, and it should be considered and definitely advised under certain circumstances. Nowadays, CML patients selected for allogeneic transplant usually have a documented resistance to 1st- and 2nd-generation TKIs or a more advanced phase of the disease, or both. The use of DLI to restore a hematologic and molecular remission is well known in CML patients relapsing after alloHSCT.[12] More recently, the use of post-transplant TKIs in combination with DLI appears to induce a rapid and durable remission even in advanced phase CML patients relapsing after alloHSCT.[13] In addition, it has been examined whether imatinib alone can delay relapse and postpone the requirement for DLI in CML patients allografted using a reduced intensity regimen. In this latter setting, imatinib was commenced on day + 35 and continued until one year after transplantation. Post-transplantation imatinib was well tolerated and abolished the risk of relapse during this period of time. For patients who relapsed after imatinib discontinuation, DLI remained highly effective in inducing a molecular remission.[14]

The risk of leukemia relapse is particularly high when the transplant is performed in Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). A prospective, randomized multicenter trial investigated the tolerability and efficacy of post-transplant imatinib administered either as prophylaxis or following detection of minimal residual

Table 1. Strategies for prevention of relapse after allogeneic hematopoietic stem cell transplantation.

	Disease application	Mechanism of action	Phase of development	Main limits
Cell therapy				
Unmanipulated DLI	AML/NHL	GvL effect	Clinical practice	GvHD
NK cells	AML	GvL effect	Ongoing	GvHD?
Combination of cell therapy and new drugs				
DLI and TKIs	CML/Ph+ALL	Synergize anti-tumor activity	Clinical practice	GvHD
DLI and 5-Aza	MDS	Enhanced GvL activity	Ongoing	GvHD?
	Reduced risk of GvHD			
	Direct cytotoxic effect			
New drugs				
TKIs	CML/Ph+ALL	Anti-tumor activity	Clinical practice	Skin, cardiovascular pleural effusion
Lenalidomide	MDS/MM	Immunomodulation and anti-tumor activity	Phase II	GvHD
Bortezomib	MM	Anti-myeloma activity	Phase II	Peripheral neuropathy
5-Aza	MDS	Cytotoxic effect and inducing tolerance	Phase II	None
Blinatumomab	BP-ALL	Anti-CD19+ immunotherapy	To be investigated	Neurological toxicity

disease (MRD). Prophylactic imatinib significantly reduced the incidence of molecular recurrence after alloHSCT compared with MRD-triggered imatinib, but the overall survival (OS) after transplantation proved equally very high (80% *vs*. 75%).[15] More recently, Shimoni *et al*. explored the use of nilotinib for the prevention of relapse after alloHSCT in advanced-phase CML and Ph+ ALL. Patients were given prophylactic nilotinib maintenance, which was started at a median of 38 days after transplantation. Some patients had to stop the treatment because of toxicities (mostly gastrointestinal and hepatic), but after nilotinib maintenance, most patients achieved or maintained a complete molecular response, and only one of them later relapsed. With a median follow up of 46 months, recipients on nilotinib maintenance showed 2-year OS and progression-free survival (PFS) rates of 69% and 56%, respectively.[16]

Toxicity of DLI

Donor lymphocyte infusion remains one of the main clinical options to salvage patients in molecular relapse after transplant or with mixed T-cell chimerism, a common event after RIC regimens which is often associated with high risk of relapse. As reported above, the use of unmanipulated or minimally manipulated donor T cells may be effective in inducing molecular remission or in conversion to full donor T-cell chimerism.[17] The main limit of this treatment is the significant GvHD-related toxicity which develops in approximately 50%-70% of the patients receiving DLI.[18] The risk of developing GvHD is dependent on the dose of DLI and the donor type, while there is no correlation with the type of disease for which DLI are given. Peggs *et al*., in a cohort of patients treated with DLI infusions for mixed chimerism or residual or progressive disease following RIC transplant, showed that GvHD was more common, occurred at lower T-cell doses, and was more severe in the unrelated donor cohort compared to sibling donor.[19] A different risk of GvHD secondary to DLI has been clearly associated with the dose of lymphocytes infused and the donor type.[20] In a recent retrospective study on 225 patients receiving transplants from HLA-matched related or unrelated donors for relapsed CML and other relapsed hematologic malignancies, multivariate analysis showed that initial DLI dose of more than $10x10^7$ CD3+/kg is associated with an increased risk of GvHD. Indeed, the cumulative incidence rates of GvHD was over 50% after a dose of DLI of more than $10x10^7$ CD3+/kg, while it was approximately 20% when the dose was less than $1x10^7$ CD3+/kg. Moreover, an initial DLI CD3+ cell dose of $10x10^7$ or higher did not decrease the risk of relapse and did not improve OS.[21] Overall, these results support the use of less than $10x10^7$CD3+ 10^3 cells/kg as the initial cell dose for treatment of relapsed hematologic disease after alloHSCT. A second important complication following DLI infusion is marrow aplasia, reported in up to 40% of patients.[22] The main factor that predicts the development of aplasia is the presence of an insufficient donor hematopoiesis before DLI.[23] Cases of aplasia are more common in patients who received DLI for hematologic relapse of CML compared to patients with AML. In patients with absence of donor residual hematopoiesis, a spontaneous recovery of marrow aplasia is unlikely, and an infusion of donor stem cells seems to be necessary to avoid the morbidity and mortality associated with prolonged aplasia. Thus, an evaluation of residual donor hematopoiesis before planning DLI treatment is recommended.

Natural killer cells and T-regulatory cells

Natural killer (NK) cells can exert a potent alloreactivity against neoplastic cells when KIR molecules on the surface of donor NK cells are not engaged by HLA class I related ligands.[24] Many clinical studies have suggested some correlation between this NK allorecognition and the outcome of allogeneic transplants, particularly in the case of haplo-identical transplant.[25] Following the experience of pilot studies,[26-28] the safety, feasibility and engraftment of haplo-identical NK-cell infusions after an immunosuppressive regimen has been tested in children with AML who had completed chemotherapy and were in first CR. After an *in vivo* lymphoid depletion with cyclophosphamide and fludarabine KIR-HLA, mismatched NK cells were infused followed by IL-2. All patients had transient engraftment for a median of ten days and a significant expansion of KIR-mismatched NK cells. Non-hematologic toxicity was limited, with no acute GvHD. All patients remained in remission, and 2-year event-free survival was 100%.[29] In another series of 30 patients who received a total of 51 NK cell enriched DLI following 3-6/6 HLA-matched, T-cell-depleted, non-myeloablative allogeneic transplantation, long-term responders had significantly improved duration of response.[30] In a phase II study including 16 patients with high-risk leukemia or multiple relapsed tumors, highly purified NK cells have been administered at days +3, +40 and +100 after haplo-identical T-cell depleted alloHSCT. Median doses of NK cells were 1.2×10^7/kg containing $0.003x10^7$ T cells/kg. Unfortunately, 4 patients developed acute GvHD of grade II or over, and in 3 out of 4 this was fatal. Acute GvHD was associated with cumulative dose of infused T cells but not with cumulative dose of infused NK cells.[31] Although it is now clear that NK-cell infusions may augment the GvL effect without significant GvHD, the expected short-term life of adoptively transferred NK cells and the laboratory work required to implement this strategy may limit a wide clinical application of this approach.

In the setting of haplo-identical transplant, another interesting field of experimental and clinical research concerns the adoptive infusion of selected T-regulatory cells (Tregs). The primary goal of these studies is aimed to manipulate the graft to reduce incidence and severity of acute GvHD (aGvHD), as well as to increase the speed and quality of immune reconstitution.[32] However, the early, post-transplant adoptive transfer of Tregs proved effective not only in improving the immune reconstitution of patients receiving an haplo-identical *ex vivo* T-cell depleted graft, but it was also associated to a low incidence of leukemia relapse in a group of patients with very high-risk AML.[33]

5-azacytidine in myelodysplastic syndromes and acute myelogenous leukemia

For MDS and AML patients, relapse usually occurs within 6-12 months after alloHSCT[34,35] and is mainly influenced by the disease risk status as defined by interna-

tional validated scores[36-39] and cytogenetic and molecular risk group.[40-43] Several pre-emptive pharmacological or immunotherapeutic approaches are theoretically possible, including hypomethylating and immunomodulating agents, or withdrawal of immunosuppression, or donor lymphocyte infusions. However, due to the absence of clinical trials comparing different approaches, the optimal treatment strategy has still not been defined.

Based on high tolerability and the possibility of administration on an outpatient basis, great interest has been shown in hypomethylating agents. A recent dose-finding study confirmed the safety (without an increase rate of GvHD) and the efficacy of low-dose 5-Aza (32 mg/m^2 per day for 5 days) to prevent relapse after alloHSCT.[44] It is worthy of note that 5-Aza, in addition to the direct cytotoxic effect, seems to accelerate the reconstitution of Tregs and induce CD8+ T-cell response to candidate tumor antigens, enhancing the GvL effect and reducing the risk of GvHD.[45-47] Therefore, considering the ability of 5-Aza in inducing tolerance by increasing the numbers of Tregs, future studies combining 5-Aza and DLI or NK cells deserve to be investigated in a prevention setting, as already carried out in patients who relapsed after alloHSCT.[48] In this regard, a study evaluating azacitidine and DLI for prevention of AML and MDS relapse is ongoing at Nantes University Hospital (*clinicaltrials.gov identifier: 01541280*).

A recent pilot study evaluated the safety and efficacy of a combination of 5-Aza (30 mg/m^2 intravenously on days 1-7) and gemtuzumab ozogamycin (3 mg/m^2 on day 8) as maintenance therapy after alloHSCT. Ten patients with high-risk AML (median age 49 years) were treated in this study. Treatment was repeated every four weeks and seems to be tolerable in the post-transplant setting, suggesting that AZA-GO might prolong survival in patients with high-risk AML.[49]

In general, considering that MDS and AML patients mainly relapse within the first year after alloHSCT, early maintenance therapy is potentially a good strategy,[50] even though this approach would imply exposing a remarkable number of already cured patients to the risks and costs of drug treatments. Thus, to partially overcome this limit, monitoring MRD may help identify patients at higher risk of relapse. Because there is usually no disease-specific molecular marker in MDS, MRD could be monitored by tracking the donor chimerism using highly sensitive molecular tools. Using this approach, Platzbecker *et al*. conducted a trial evaluating the efficacy of a pre-emptive treatment with 5-Aza for MRD that was decided on the basis of a decreasing CD34-donor chimerism. These results showed an initial response in 80% of patients, with 20% in continued MRD-negative remission after the administration of a limited number of 5Aza cycles.[51]

Lenalidomide in myelodysplastic syndromes and multiple myeloma

The ability of lenalidomide to activate on T and NK cells is well known and has been demonstrated in previous studies.[52] More recently, it has been found that lenalidomide has the potential to increase the effector function of T cells and can reverse T-cell tolerance in MDS patients. Based on these immunomodulatory properties, with the hope of increasing the GvL effect following alloHSCT, lenalidomide has been tested as pre-emptive treatment in both myeloma and MDS patients early after transplantation. Unfortunately, a phase II study evaluating lenalidomide as maintenance therapy after alloHSCT in patients with AML or MDS showed an increased risk of GvHD.[53] These poor results are in keeping with those observed in patients with MM in whom the early administration of this drug (within 6 months after alloHSCT) was associated with a high rate of severe aGvHD.[54]

Bortezomib in multiple myeloma

The use of bortezomib has shown important anti-myeloma activity in both the relapsed/refractory and the up-front setting. However, due to its side-effects, such as peripheral neuropathy, its use after alloHSCT could prove difficult. Kroger *et al*. reported[55] the effect of DLI alone or in combination with the new agents thalidomide, bortezomib, and lenalidomide in 32 patients with MM who achieved only partial remission or very good partial remission after alloHSCT. The results of this study showed that 59% of patients achieved CR, and this CR was of clinical relevance with a significantly improved progression-free survival (PFS) at five years. A phase II study is ongoing to evaluate whether bortezomib administration after non-myeloablative alloHSCT in high-risk myeloma patients might improve the outcome of these patients (*clinicaltrials.gov identifier: 02308280*). Another phase II, open-label, multicenter, non-randomized study is ongoing to evaluate the role of MLN9708, an orally bioavailable, potent, reversible inhibitor of the 20S proteasome, that was well tolerated with minimal peripheral neuropathy[56] as maintenance after alloHSCT in patients with high-risk MM (*clinicaltrials.gov identifier: 02168101*).

Future perspectives: the use of bispecific antibodies and chimeric antigen receptor T cells

A significant role for MRD was first observed in ALL. Several studies performed in children[57,58] and in adults[59-61] have clearly shown that MRD measurement is an independent prognostic factor for poor outcome. Patients with persistent MRD during induction and consolidation of front-line treatment, or who become MRD-positive following treatment, have a poor leukemia-free survival. Recent results showed that also patients undergoing alloHSCT with measurable level of MRD clearly show an inferior outcome after transplant due to a significant increase in risk of leukemia relapse.[62-65] The evidence in our experience of an adverse relationship between measurable level of MRD before transplant and outcome after alloHSCT is highlighted in Figure 1. This implies that, whenever possible, in order to prevent the risk of relapse, patients selected for allogeneic transplant should be considered for newer experimental treatment strategies able to achieve a complete molecular remission. Among such innovative treatments, blinatumomab, the first member of a novel class of T-cell engaging, bispecific single chain (BiTE) antibodies (engaging T cells for re-directed lysis of CD19+ target cells) showed very encouraging results in terms of high percentage of MRD response rate. This

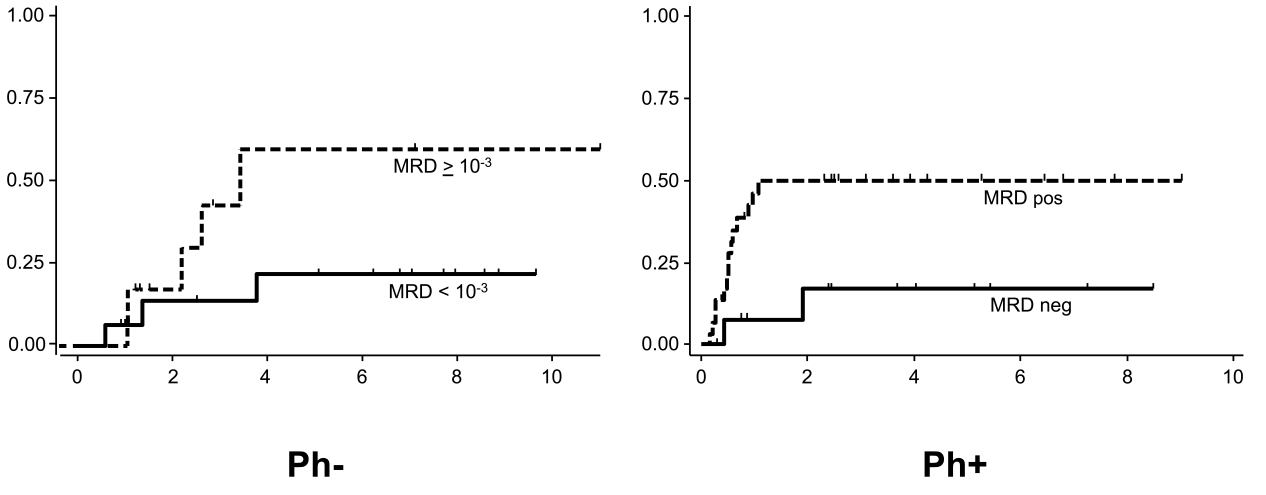

Figure 1. Incidence of relapse after allogeneic hematopoietic stem cell transplantation in adult acute lymphoblastic leukemia (ALL) by minimal residual disease status at conditioning. Patients were treated according to the Northern Italy Leukemia Group (NILG) ALL clinical trials.[59,78]

translates into a favorable relapse-free survival (RFS).[66,67] Indeed, the first pilot trial conducted in MRD+ ALL resulted in 80% of patients achieving MRD negativity. Nine of 20 patients received alloHSCT after blinatumomab treatment and 65% of these were in hematologic CR at a median follow up of 33 months. According to these promising results, and given its manageable toxicity profile,[68] a short period of treatment with blinatumomab as pre-emptive therapy before transplant could have the potential to achieve a convincing complete molecular remission and, thus, to promote an improved cure rate after transplantation. This hypothesis needs to be evaluated and warrants the planning of specific *ad hoc* designed clinical trials. The concept that persistence or becoming MRD positive after alloHSCT can herald overt hematologic relapse[57-59,69-71] has been clearly demonstrated. Therefore, careful monitoring of MRD after alloHSCT in ALL patients is very important because it may allow for rapid preventive intervention. In this context, the use of blinatumomab may be more attractive than conventional treatments, such as tapering or withdrawal of immunosuppressive therapy or use of DLI.

Another area of investigation could be represented by the use of CD19-directed chimeric antigen receptor (CAR)-modified T-cell therapies that have shown exciting results in highly refractory populations.[72,73] However, the clinical use of these cells as a pre-emptive strategy of leukemia relapse after alloHSCT should take into account the costs and the high technical complexity for the clinical grade manufacturing of these genetically modified cells, as well as the important levels of toxicity, mainly related to the massive cytokine release following their *in vivo* infusion.[72,74]

Based on the success of (CAR) T cells in B-cell precursor ALL and B-cell chronic lymphocytic leukemia, similar approaches are currently being investigated with the hope of targeting also myeloid malignancies. The project to target CD123, the interleukin-3 receptor α-chain, which is over-expressed in AML compared with normal hematopoietic stem cells is of interest in this regard. CARs containing a CD123-specific single chain variable fragment, in combination with a CD28 co-stimulatory domain and CD3-ζ signaling domain, have been generated and CD123-CAR re-directed T cells mediated potent effector activity against CD123+ AML cells.[75,76] The possibility of developing a clinical approach using these cells is currently under evaluation.[77]

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