Hematopoietic stem cell transplantation for chronic lymphocytic leukemia

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A B S T R A C T

In spite of important advances in therapy, chronic lymphocytic leukemia (CLL) remains an incurable disease. Patients refractory to chemoimmunotherapy or with a response shorter than 24-36 months have a very poor outcome. Currently, patients unlikely to respond to therapy may be reasonably identified thanks to several biomarkers (*e.g., TP53* deletions and/or mutations). Non-myeloablative allogeneic transplants result in a sustained complete remission (CR) in around 50% of patients, including those with poor prognosis indicators. Non-relapse mortality (16-23%) after this procedure, mainly due to infections and graft versus host disease, is of concern. Disease status at transplantation (CR *vs.* <CR) highly determines the success of the transplant. New biological agents targeting specific pathways of CLL pathophysiology (*e.g.*, B-cell receptor kinase inhibitors, Bcl-2 family inhibitors, cyclin-dependent kinases) might be useful to reduce the tumor mass before transplant. Finally, based on the pronounced sensitivity of CLL to immunomodulation, a variety of cell therapies (T- and NK-cells based) are currently being investigated.

Introduction

In the last two decades, important progress has been made in the treatment of patients with chronic lymphocytic leukemia (CLL). The introduction of purine analogs and monoclonal antibodies in the treatment of this form of leukemia has resulted in an important increase in the complete response (CR) rate (including MRD-negative CRs) and progression-free survival (PFS). Of note, there is proof that chemoimmunotherapy, that is, fludarabine, cyclophosphamide, rituximab (FCR), prolongs the overall survival (OS) of patients with this disease. Because of this, FCR is the new gold-standard for the treatment of CLL.[1,2] Unfortunately, there is a fraction of patients, ranging from 10-20% in treatment-naïve cases to 30-50% in advanced, multi-treated cases, who are resistant to modern therapy. In addition, the high proportion of CRs achieved with chemoimmunotherapy does not translate into the cure of the disease, since patients experience disease progression and die from CLL or treatment-related complications.

The prognosis of subjects with CLL is extremely variable. While there are patients whose life expectancy is not different from that of the general population, others have a rapidly fatal course. New prognostic factors and response predictors identified over the last years allow us to identify patients with aggressive disease and poor prognosis.[3] Thus, patients with *TP53* deletions and/or mutationsaberrations, refractory to fludarabine-based therapy or with short (<24-36 months) PFS after chemoimmunotherapy have a very poor outlook (median survival inferior to 2 years).[4-6] Unfortunately, current treatments only allow to control the disease temporarily.[7] Due to these reasons, the number of stem cell transplants, and particularly allogeneic stem cell transplantation, in patients with CLL has been rapidly increasing[8] (Figure 1).

In this article, the role of stem cell transplantation in the treatment of CLL is reviewed, with special focus on allotransplants.

Autologous stem cell transplantation

The first studies on autologous transplants in CLL were published in the 1990s but only recently, thanks to a number of controlled studies, has the potential role of this treatment modality as currently conceived been largely clarified.

Three randomized trials (Table 1)[9-11] have mainly confirmed previous notions derived from phase II studies:[12] i) non-relapse mortality (NRM) is less than 10%; ii) the clinical benefits (*i.e.*, longer PFS) are limited to patients without unfavorable prognostic factors and with responsive disease; iii) results are better in patients transplanted early in the course of the disease;[8,13] iv) the constant pattern of relapse indicates that autologous transplants do not cure the disease;[14-18] v) the impact of poor prognostic biomarkers (*e.g.*, del 17p) on patients' outcome is not overcome by autografts;[19,20] vi) autologous transplants are not effective as salvage therapy of refractory disease;[3,13,21] vii) autografted patients may develop secondary myelodysplasia/acute leukemia, which seems to be related to prior exposure to purine analogues, low cell-dose

infused and transplant conditioning regimen (*i.e.*, total body irradiation (TBI) and cyclophosphamide);[16,22] viii) stem cell harvesting may be difficult, or impossible, in patients treated with purine analogs.[23]

Altogether, these results are not surprising if one considers that the therapeutic effect of autologous stem cell transplantation relies on chemo (radio)-therapy only.

It could be argued that treatment administered to patients included in randomized trials comparing autologous transplants *versus* conventional therapy was suboptimal by current standards. In this regard, a recent retrospective analysis comparing the fate of patients treated with FCR and that of patients receiving autologous transplantation has failed to find major differences in patients' outcomes.[24] Furthermore, given the excellent results currently obtained with chemoimmunotherapy and their continuous improvement, it is difficult to foresee new randomized trials on autologous stem cell transplantation, at least as devised up to now. In fact, the decreasing enthusiasm of physicians toward autologous transplantation (or

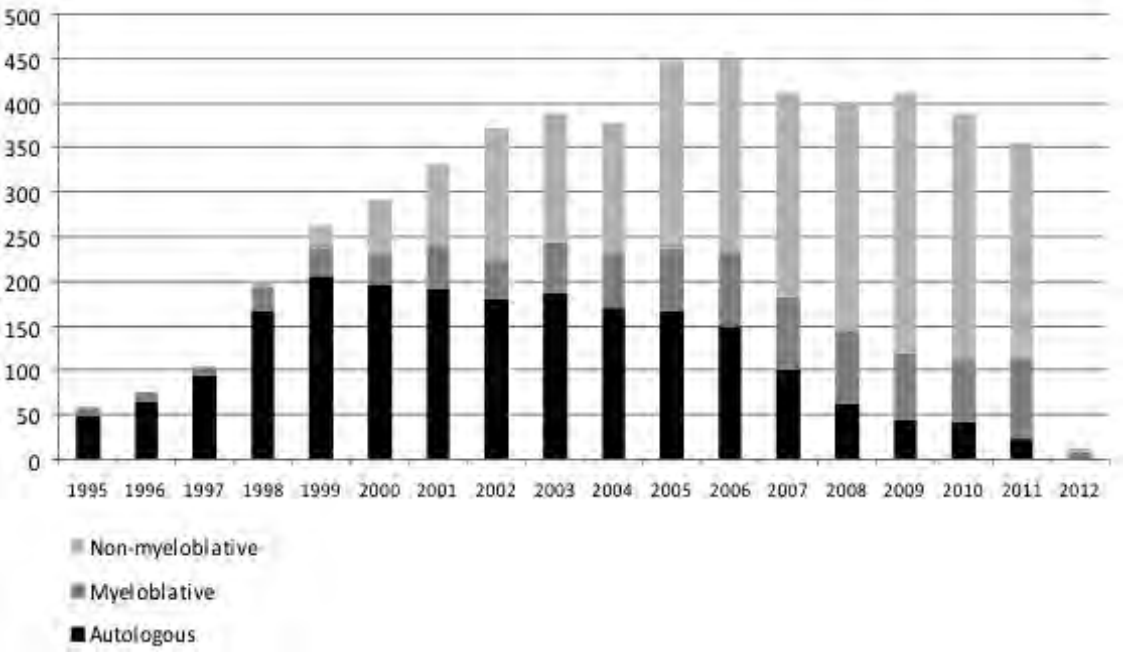

Figure 1. Stem cell transplants for CLL: transplant modality (EBMT, 1995-2012).

Table 1. Prospective randomized clinical trials comparing autologous stem cell transplants with conventional chemotherapy for CLL.

Phase III trials	Induction therapy	Status of the disease (before randomization)	Treatment	N	NRM (%)	PFS(%)	OS(%)
Michallet *et al.*[9] 2011	Several[a]	59% CR 41% less than CR[b]	SCT *vs.* observation	112 111	4 0	42 (5-yr) 24 (5-yr)	85.5 (5-yr) 84 (5-yr)
Sutton *et al.*[10] 2011[c]	Mini-CHOP x 3c+	44.6% CR	SCT *vs.* observation	52 53	NR	79.8 (3-yr) 35.5 (3-yr)	95.7 (3-yr) 97.8 (3-yr)
	Fludarabine x 3c	55.4% nonCR	SCT *vs.* FC	46 48	NR	48.9 (3-yr) 44.4 (3-yr)	81.7 (3-yr) 87 (3-yr)
Brion *et al.*[11] 2011[d]	CHOP x6c CHOPx 3c	NA	CHOP or F *vs.* SCT	39 43	NR	104.7 m 107.4 m	22 m 55.3 m

CR: complete response; N: number of patients; NRM: non-relapse-mortality; EFS: event free survival; OS: overall survival; NR: not reported; NA: not applicable; m: months; FC: fludarabine and cyclophosphamide; F: fludarabine; CHOP: cyclophosphamide, vincristine, doxorubicin and prednisone; SCT: stem cell transplan; yr: year. [a]Induction therapies included fludarabine combinations and alkylating agents. Only 9 patients received purine analogues combinations and rituximab. [b]Included very good partial response (VGPR) and nodularPR. [c]99 patients from the CR were simultaneously registered for the EBMT trial. [d]In this study previously untreated patients were randomized from induction therapy.

their higher confidence on chemoimmunotherapy), was a major problem for the completion of the above-mentioned randomized studies.

Allogeneic stem cell transplantation

Myeloablative conditioning transplants

Historically, the first allogeneic stem cell transplants performed in patients with CLL were after fully myeloablative conditioning, with cyclophospamide (Cy) and TBI being the most commonly administered regimen. Although the best conditioning regimen has never been formally investigated, two studies showed a detrimental effect by adding VP-16 to Cy plus TBI or to Cy plus Busulfan.[25,26]

The results from the most relevant series of patients with CLL treated with myeloablative conditioning allogeneic stem cell transplants are summarized in Table 2.[15,16,25-32] These studies show: i) a high non-relapse mortality (NRM), ranging from 24% in single center studies to 47% in multicenter and registry series; ii) DFS and OS plateaus of 40-60% at 4-5 years from transplantation; iii) relapse rate of 2-25% at 3-5 years after transplant, with infrequent relapses beyond 4 years from the procedure except in the case of T-cell depleted grafts;[16] iv) incidence of acute graft *versus* host disease (aGVHD) between 49-56% and that of chronic GVHD (cGVHD) above 50%; v) sustained CR even in patients refractory to treatment, including purine-analogs based; vi) overcoming the negative impact of poor prognostic markers, such as deletion 17p or unmutated *IGHV* genes.[20,33]

The differences in treatment results of allogeneic compared with autologous transplants are mainly due to their mechanism of action. While in autologous transplants, the treatment effect is based on the high-dose preparative regimen only, in allogeneic stem cell transplantation, the graft *versus* leukemia (GVL) is essential to eradicate the disease. The existence of a powerful GVL effect is supported by several observations: the slow disappearance of malignant cells from bone marrow and peripheral blood that remained after conditioning, the progressive resolution of organomegaly, and the conversion from a minimal residual disease (MRD)-positive to a MRD-negative status. Notably, these events may require several months following transplantation to take place and frequently triggered by the development of GVHD. Furthermore, as in other hematologic malignancies, cGVHD decreases the risk of relapse and donor-lymphocyte infusions (DLI) may control the disease in case of no response or relapse.[34-37] Likewise, the T-cell depletion of the graft results in a high, constant relapse rate,[16] and a small series of transplants from identical twins have shown results comparable to those obtained with autologous transplants.[38]

Reduced intensity conditioning transplants

Fully myeloablative allogeneic stem cell transplantation in CLL is limited by two major facts: i) the high NRM due to GVHD and infections, and ii) the advanced age of most patients with CLL, with a median age at diagnosis of 72 years. In fact, more than 80% of patients with CLL are older than 50,[39] the upper age limit in most centers for allogeneic stem cell transplantation. Because of this and to the sensitivity of CLL to immunomodulatory mechanisms (*i.e.*, GVL effect), allogeneic transplantation using non-myeloablative conditioning regimens is considered as the transplant modality of choice in this leukemia.

Table 2. Myeloablative conditioning allogeneic stem cell transplants for CLL.

Study	Patients (no.)	Conditioning regimen	Refractory patients(%)	Unresponsive disease pre-SCT (%)	Characteristics of the study	NRM (%)	Relapse risk (%)	PFS (%)	OS (%)	aGVHD (II-IV) (%)	cGVHD (%)
Pavletic *et al.*[25] 2000	23	Cy+TBI±VP-16	61	60	Single-center series	30	2 (5-yr)	62 (5-yr)	65 (5-yr)	54	68
Michallet *et al.*[27] 2001	209	Several	44	–	Registry study	40	25 (3-yr)	NR	55 (3-yr)	–	–
Horowitz *et al.*[28] 2000	242	Several	50	–	Registry study	30	25 (3-yr)	44 (3-yr)	45 (3-yr)	–	–
Khouri *et al.*[29] 2002	28	Cy+TBI/BEAM(n=1)	68	68	Single-center series	NR	NR	26 (5-yr)[a]	31 (5-yr)[b]	49	64
Doney *et al.*[26] 2002	25	Bu+Cy Cy+TBI±VP-16	64	76	Single-center series	40	<10	NR	32 (5-yr)	56	55
Esteve *et al.*[15] 2001	46	Several	55	–	Multicenter study	41	18 (5-yr)	51 (5-yr)	58 (5-yr)	–	–
Michallet *et al.*[30] 2003	54	Several	85	–	Registry study	46	NR	37 (10-yr)	41 (10-yr)	–	–
Esteve *et al.*[31] 2005	17	Cy+TBI	35	–	Single-center series	24	9 (6-yr)	66 (6-yr)	76 (6-yr)	–	–
Gribben *et al.*[16] 2005	27	Cy+TBI+TCD	0	0	Single-center series	27	68 (6-yr)	24 (6-yr)	58 (6-yr)	–	50
Toze *et al.*[32] 2005	30[b]	Cy+TBI±other Bu+Cy	NR	NR	Two-center series	47	19	39 (5 yr)	39 (5yr)	52	65

NRM: non-relapse mortality; PFS: progression-free survival; OS: overall survival; NR: not reported;Cy: cyclophosphamide; TBI: total body irradiation;TCD: T cell depletion; Bu: busulfan; a and cGVHD: acute and chronic graft versus host disease; SCT: stem cell transplant; yr: year.
[a]Referred to the subset of refractory patients. [b]Ten of 30 patients received an unrelated transplant.

A variety of reduced intensity conditioning regimens is used, usually consisting of fludarabine (because of its cytotoxic and immunosuppressive effect) combined with one of the following partners: low dose TBI, cyclophosphamide, busulfan, or melphalan with monoclonal antibodies (*i.e.*, rituximab, alemtuzumab) sometimes added to the conditioning regimen. In all, these conditioning strategies shift from predominantly myeloablative to mainly immunosuppressive regimens.[40] Unfortunately, there is no widely accepted conditioning regimen, with different combinations (in most cases with only slight, minor modifications) being used at different institutions.

The main results of the four largest series of non-myeloablative transplants in CLL are summarized in Table 3A.[41,42-45] Collectively these studies show: i) NRM inferior to 23% (15-23% at 1 and 3 years); ii) PFS of 36-54% at 4-5 years after transplant, and a survival plateau of 50%; iii) relapse risk of 30-40% at 5 years; iv) incidence of aGVHD and cGVHD of 34-45% and 51-64%, respectively; 5) effectiveness in overcoming the negative impact of poor prognostic markers (*i.e.*, *17p* deletion, unmutated *IGHV*, purine analogs refractoriness), underscoring the importance of the GVL effect.[41,46,47]

Age, disease status, number of lines of therapy before transplantation, interval diagnosis-transplant, acute GVHD, HLA disparities, sex mis-matching, and patients' comorbidities have all been found to be prognostically relevant in allotransplants as a whole.[15,16,25,26,28-32,48] As far as non-myeloablative allotransplantation is concerned, it is not surprising that the status of the disease immediately before transplant (CR *vs.* <CR) has been found to be an important prognostic factor in multivariate analysis for all relevant end-points (*i.e.*, NRM, PFS, OS) (Table 3B). Other variables with independent prognostic value are lymphadenopathy size (≥5 cm), T-cell depletion of the graft, or alemtuzumab exposure prior to stem cell transplant and patients' comorbidities.[41-45,49] In turn, achieving MRD negative status within the first year of transplant is a strong predictor for durable disease control.[41]

GVHD and related complications are the main causes of NRM. As stated earlier, the incidence of cGVHD reported in non-myeloablative stem cell transplant (51-64%) does not differ from that observed after myeloablative regimens (50-68%) (Tables 2 and 3A).

Allogeneic transplants from unrelated donors

Following the general trend for allogeneic stem transplantation, the use of unrelated donors in patients with CLL has been increasing over the last years (Figure 2). In the most recent series, the percentage of patients with CLL receiving non-myeloablative transplants from unrelated donors ranges from 37-67% (Table 3A).[35,43-46] Of note, donor type (related *vs.* unrelated) does not appear to influence patients' outcome. In fact, there are signals toward slightly better results in patients receiving unrelated transplants, possibly due to a stronger GVL effect.[42,50]

Treatment results in patients with CLL treated with allogeneic stem cell transplantation (myeloablative and non-myeloablative) from unrelated donors are summarized in Table 4.[32,42,48,51,52] Overall, results for myeloablative and non-myeloablative conditioning regimens are as follows: i) NRM rate of 38% *vs.* 24-28% at 2-5 years after transplant; ii) PFS between 10% and 30% *vs.* 51% at 5 years; iii) OS of 20-59% *vs.* 48-51% at 5 years; iv) relapse risk of around 30% in both modalities; v) incidence of aGVHD 45-52% *vs.* 28-87% and of cGVHD 66-85% *vs.* 31-65%, respectively.

There is no or little experience with other types of transplants (*e.g.*, haploidentical, cord blood). However, two preliminary reports suggest that cord-blood might be an alternative hematopoietic stem cell source for patients lacking either a sibling or unrelated donor;[53,54] however, the slow, delayed immune recovery after cord-blood transplants is of concern.

A few retrospective studies have analyzed the relative merits of non-myeloablative *versus* myeloablative transplants. The main conclusion is that in non-myeloablative

Table 3A. Reduced intensity conditioning allogeneic stem cell transplants for CLL.

Study	Patients (no.)	Median age (range)	Purine analogues refractory (%)	Unresponsive disease pre-SCT(%)	NRM (%)	Conditioning regimen	Donor (%)	RR (%)	PFS (%)	OS (%)	aGVHD II-IV (%)	cGVHD
Sorror *et al.*[42] 2008	82	55 (42-69)	87	56	23 (5-yr)	Fluda±TBI (2 Gy)	Rel - 63%	38 (5-yr)	39 (5-yr)	50 (5-yr)	39 *vs.* 43 related *vs.* unrelated	49 *vs.* 53 (5-yr) related *vs.* unrelated
Khouri *et al.*[43] 2011	86	58 (36-70)	83	17	17 (1-yr)	Fluda+Cy± Rituximab	Rel - 50%	39 (3-yr)	36 (5-yr)	51 (5-yr)	37	56 (5-yr)
Dreger *et al.*[41] 2010	90	53 (27-65)	47	21	23 (4-yr)	Fluda+Cy-based± ATG±TCD[a]	Rel - 40%	40 (4-yr)	42 (4-yr)[b]	65 (4-yr)	45	55 (2-yr)
Brown *et al.*[44,45] 2008[c]	62	55 (36-55)	NR	56	15 (4-yr)	Fluda +Bu	Rel - 33%	30 (3-yr)	54 (3-yr)	65 (3-yr)	34	64 (2-yr)

NRM: non-relpase-mortality; RR: risk of relaspse; PFS: progression-free survival; OS: overall survival; Cy: cyclophosphamide; TBI: total body irradiation; Rel: related donor; Bu: busulfan; aGVHD: acute graft versus host disease; cGVHD: chronic graft versus host disease; yr: year.

[a]TCD, T cell depletion was performed in 12 patients. [b]This % refers to event free survival. [c]Update of the series published in 2006, presented at the American Society of Hematology in 2008 (88 patients included, 62 received a non-myeloablative transplant).

transplants, the lower NRM is counterbalanced by a higher risk of relapse, making patients' outcomes eventually similar with the two procedures.[50,55] Although based on these results, it could be argued whether there is still room for myeloablative conditioning in selected young patients with CLL refractory to treatment, the fact is that myeloablative transplants in CLL have been virtually abandoned. Thus, the EBMT registry shows that the number of myeloablative conditioning transplants has significantly decreased over the last years whereas that of reduced intensity transplants is increasing (Figure 1).[56] A case control comparison of standard therapy with non-myeloablative allotransplants in high-risk patients with CLL has shown a survival benefit for allografted patients.[57]

Table 3B. Predictors factors for NRM, RR, PFS and OS after reduced intensity conditioning allogeneic stem cell transplants.

Study	Patients (no.)	Conditioning regimen	NRM	RR	PFS	OS
Sorror *et al.*[42,49] 2008[a]	82	Fluda±TBI (2 Gy)	HCT-CI score	HCT-CI score Refractory disease Prior alemtuzumab 12 m[a]	Bulky disease (≥5cm) HCT-CI score	Bulky disease (≥5cm) HCT-CI score
Khouri *et al.*[43] 2011	86	Fluda+ Cy±Rituximab	Below normal levels of IgG	Refractory disease Donor chimerism ≤90% by day 90	HLA-A1-/HLA-A2+/HLAB44+ Donor chimerism ≤90% by day 90 Marrow graft source	CD4< 100/mm^3 Below normal levels of IgG
Dreger *et al.*[41] 2010	90	Fluda+Cy-based± ATG	Refractory disease Alemtuzumab TCD ECOG of 1	MRD positivity at 1 yr after transplant Age	Refractory disease[b] Alemtuzumab TCD Age	Refractory disease Alemtuzumab TCD
Brown *et al.*[44] 2006	46	Fluda+Bu	Refractory disease	Refractory disease	N. lines therapy BM involvement	N. lines therapy BM involvement Refractory disease[b]

Fluda: fludarabine; TBI: total body irradiation; Cy: cyclophosphamide; Bu: busulfan; NRM: non-relapse mortality; RR: relapse risk; PFS: progression free survival; OS: overall survival; HCT-CI: hematopoietic stem cell transplant-comorbidity index; MRD: minimal residual disease; TCD: T cell depletion; BM: bone marrow.
[a]From an update presented at the American Society of Hematology (ASH) 2010 (n=136). [b]From an update presented at the American Society of Hematology (ASH) 2008 (n=62)[45]

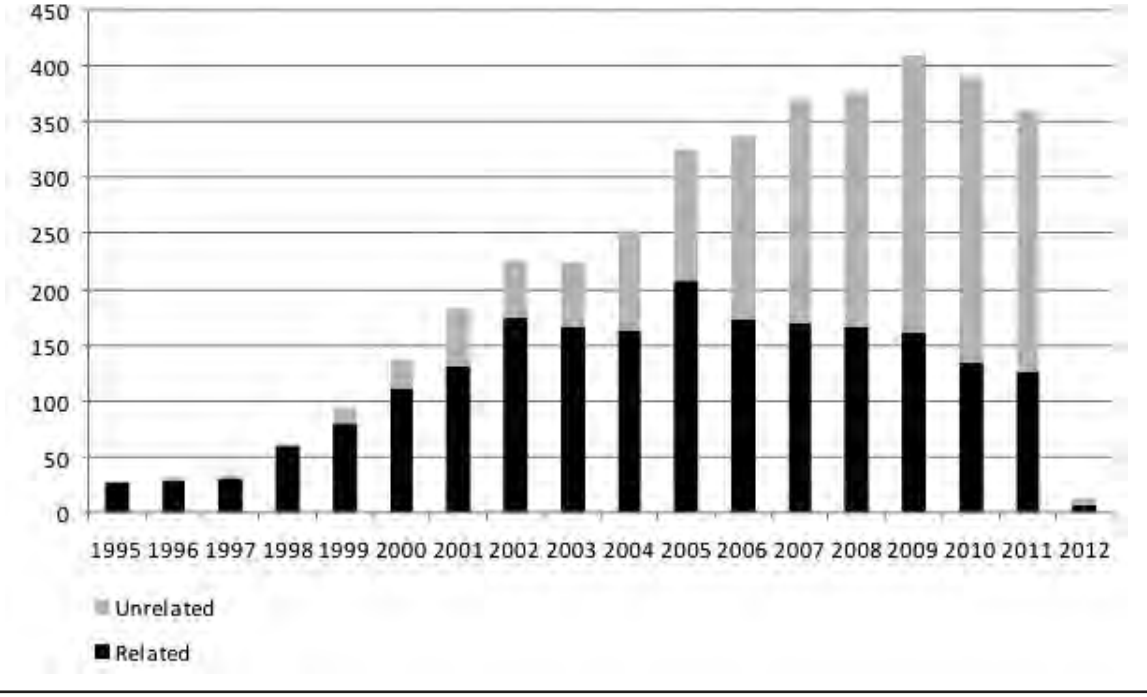

Figure 2. Allotransplants for CLL: donor type (EBMT, 1995-2012).

Table 4. Unrelated donor allogeneic stem cell transplants for CLL.

Study	Patients (no.)	Refractory patients (%)	NRM (%)	Conditioning regimen	RR (%)	PFS (%)	OS (%)	aGVHD (II-IV) (%)	cGVHD (%)
Schetelig *et al.*[51] 2003	15	-	28 (2-yr)	Fluda + Bu + ATG		67 (2-yr)	72 (2-yr)	87	31
Michallet M *et al.*[48] 2005	214	-	-	Several	-	-	59 (3-yr) Myelo-ablative	49	66
							48 (3-yr) Non-myeloablative	28	63
Pavletic *et al.*[52] 2005	38	55	38 (5-yr)	Cy+TBI ±others	32 (5-yr)	30 (5-yr)	33 (5-yr)	45	85
Toze *et al.*[32] 2005	10	NR	NR[a]	Several	NR	10 (5-yr)	20 (5-yr)	52	NR
Sorror *et al.*[42] 2008	30	87	24 (5-yr)	Fluda + TBI (2 Gy)	26 (5-yr)	51 (5-yr)	51 (5-yr)	43	53

NRM: non-relapse mortality; RR: risk of relapse; PFS: progression-free survival; OS: overall survival; Fluda: fludarabine; Bu: busulfan; ATG: antithymocyte globulin; TBI: total body irradiation; Cy: cyclophosphamide;Bu: busulfan;aGVHD: acute graft versus host disease; cGVHD:chronic graft versus host disease; NR: not reported; yr: year
[a]Unrelated donor was associated with NRM in univariate and multivariate analysis.

Transplant recommendations

In 2007, the EBMT proposed recommendations for allogeneic stem cell transplantation in CLL, these including; i) non-response or early relapse (within 12 months) after purine analogues treatment; ii) relapse within 24 months after having achieved a response with purine-analogue-based combination therapy or autologous transplantation; and iii) patients with P53 abnormalities requiring treatment.[8] Although these recommendations have not been formally revised, the evidence accumulated since their publication strongly supports their validity. However, mechanisms accounting for resistance to treatment are unfolding in a more complex way than initially thought. Recently, new mutations related to fludarabine-refractoriness (*e.g.*, NOTCH-1, SF3B1, BIRC3), not necessarily overlapping with *TP53* aberrations, have been described.[58-62] But these mutations are not yet routinely studied, and it remains to be seen whether they will consolidate as response predictors.

Finally, an important practical point is that in those cases with a clear indication for allotransplantation, the procedure should not be deferred.

New trends to improve transplant results

Challenges to improve the outcome of patients with CLL receiving allogeneic stem cell transplantation include: i) to find more effective treatments for refractory CLL, and ii) to make transplantation safer and more effective.

New treatments

Since the outcome of patients with CLL treated with allogeneic stem cell transplantation depends highly on the disease status, the first goal before proceeding to transplantation is to achieve the highest possible reduction in the tumor mass, and, if possible at all, a CR. Unfortunately, there are no good salvage therapies for patients with CLL refractory to chemoimmunotherapy. Although alemtuzumab or rituximab plus high-dose steroids may produce transient responses, the immunosuppressive effect of these regimens is of concern.[63,64] A frequent approach is to use a lymphoma-type regimen (*e.g.*, R-CHOP, R-ICE, R-ESHAP) to control the disease until the transplant is feasible.

Progress in the knowledge of the biology of CLL is allowing the development of new agents, which target disease pathways involved in the pathogenesis of the disease. Compounds with proved effectiveness and already in clinical use are ofatumumab, bendamustine, and lenalidomide, particularly in combination with other drugs.[65-68] Other agents in relatively advanced phases of development are GA-101 (an anti-D20 monoclonal antibody),[69] cyclin-dependent kynase inhibitors (*e.g.*, flavopiridol, dinaciclib),[70,71] BCR-signal transduction inhibitors (*e.g.*, CAL-101, PCI 32765),[72,73] and anti-BCL2 molecules (*e.g.*, ABT-26 or Navitoclax).[74] CAL-101 and PCI-32765 offer special promise since they are effective in refractory disease and in reducing bulky lymph nodes.

Making transplants safer and more effective

Besides general aspects (*e.g.*, age, sex, parity, CMV serologic status, ABO groups), the HLA donor-patient compatibility is very important, with patients highly matched to their donor having better outcomes. Nowadays, DNA-based techniques are routinely employed, permitting molecular typing. In those cases in which molecular (allele-based) typing is not available, serological (antigen-based) typing can separate transplants into well-matched, partially matched, and mismatched.[75] A retrospective analysis from the EBMT showed that there were no differences in the prognosis of patients transplanted from a sibling or a well-matched unrelated donor, while patients receiving a mismatched or partially matched transplant had a poorer outcome.[76]

A question to be kept in mind is the possibility that in

peripheral blood, the HLA-compatible donor harbors a monoclonal B cell population immunophenotypically identical to that of CLL (Monoclonal B cell lymphocytosis), which can be detected in up to 12% of the first-degree relatives of patients with CLL and in up to 10% in healthy population older than 59.[77,78] Although the potential risk of transmission of MBL clones to patients is of concern, only a few cases of CLL after allogeneic stem cell transplantation have been reported.[79,80,81]

Assessing pretransplant comobordities is of great importance. The hematopoietic stem cell transplantation-comorbidity index (HCT-CI) adapted from the Charlson index (CCI) allows us to classify patients into three groups with different transplant-risk.[82] Similarly, the EBMT risk score that takes into account patient's age, disease status, time from diagnosis to transplant, donor type, and donor-recipient gender is also useful to evaluate the risk of the procedure.[76,83] These indexes can be of help when discussing the pros and cons of allogeneic stem cell transplantation as a treatment option.

Increasing the GVL effect without triggering, or making worse, GVHD is an old challenge in allogeneic stem cell transplantation. Rituximab seems to promote the presentation of cell-derived peptides by antigen-presenting dendritic cells, and facilitate the generation of specific donor-deriven cytotoxic T cells, which may enhance the GVL effect. The MD Anderson group has suggested that giving rituximab before and after transplantation results in a higher response rate and a lower incidence of cGVHD.[84] In line with this notion, an ongoing CALGB study (CALGB 100701/NCT01027000), in which rituximab is given along with either fludarabine and busulfan or cyclophospamide, followed by rituximab maintenance therapy up to 12 months after transplant, has to determine whether this strategy improves PFS as a main objective. The same topic is being studied in a phase II study (NCT00104858) in which the combination investigated consists of fludarabine, low dose TBI, and rituximab given before and after transplant (six total doses). In the same line, the GETH-CLL4 (Grupo Español de Trasplante Hematopoyético) is investigating the addition of five doses of ofatumumab, a second-generation anti-CD20 monoclonal antibody, to fludarabine and melphalan in patients with high-risk CLL submitted to non-myeloablative transplants (NCT01455051). This study also envisages the possibility of giving ofatumumab if donor lymphocyte infusions (DLI) are required within 3 years after transplant.

Treatment of disease relapse presents important difficulties. A few reports on the use of DLI in that setting show poor results particularly in case of clinically overt relapse. In addition, DLI can lead to severe GVHD. Nevertheless, in a few studies the combination of DLI with immunmodulatory agents (*i.e.*, rituximab) has resulted in better results, with long lasting CRs reported in around 50% of the patients.[42,43] Immunomodulatory interventions as pre-emptive therapy are extremely appealing since they may prevent clinical relapse. Indeed, there is some indication that in patients with increasing MRD levels after transplant, which are likely to relapse, such immunomodulatory interventions, can induce MRD negativity. Further studies are needed to establish in which patients and when pre-emptive therapy might be specially useful.[37,41,85] Other approaches aimed at preventing relapse include the use of immunomodulatoy agents as maintenance therapy after transplantation.

GVHD is an important cause of NRM mortality. The strategies most widely investigated to decrease GVHD have been based on T-cell depletion of the graft by using either *in vitro* T-cell antibodies (such as ATG) or *in vitro* and *in vivo* use of alemtuzumab. Of note, alemtuzumab decreases the incidence of cGVHD but endangers the outcome of allotransplanted patients due to graft failure, disease relapse, and infectious complications (especially viral infections).[41,44,86,87] It has been reported that DLI in patients who relapse after a T cell depleted graft results in a similar incidence of cGVHD and PFS than unmanipulated grafts.[88]

Beyond stem cell transplantation

Immunoregulatory mechanisms play an important role in controlling cancer. CLL is not an exception to this rule. The results of allotransplantation in patients with CLL demonstrate clearly that the durable eradication of MRD is only possible through the GVL effect. Against this backdrop, an interesting approach is to use different modalities of T-cell and NK-cell therapy in the treatment of patients with CLL (and other forms of B-cell malignancies). In this regard, CLL cells transduced to CD154 can induce an autologous antigen-specific T-cell response. Preliminary results of a phase Ib trial showed that Ad-ISF35-Transduced autologous cells in combination with FCR induces complete and partial responses in patients with refractory CLL and/or with a *p53* defective pathway.[89]

Another approach consists of using a retroviral vector that encodes an anti-CD19 chimeric antigen receptor (CAR) that recognizes the CD19 antigen and can be used to mediate genetic transfer of this CAR to T cells. In vitro studies showed that anti-CD19-CAR-transduced T cells could specifically recognize and kill primary CLL cells by secreting IFN-γ and IL-2.[90] Seemingly, clinical benefit has been observed in patients in which autologus T cells are infused after giving a conditioning regimen including cyclophosphamide.[91] A phase I/II trial investigates the safety and toxicity of post-transplant treatment with donor T cells engineered to target CD19 in patients who have had a matched related allogeneic hematopoietic stem cell transplant for a CD19+ B cell malignancy and at high-risk of disease relapse after transplant (NCT00924326).

Conclusions

The prognosis of patients with CLL failing to respond to modern therapy is extremely poor, their survival being measured in months. Reduced intensity conditioning allogeneic transplantation may overcome the negative impact of poor prognostic factors (*i.e.*, *TP53* deletions and/or mutations, chemoimmunotherapy refractoriness) in CLL. The success of transplantation depends highly on the chemosensitivity of the disease. Because of this, allotransplantation should be considered in physically fit patients with CLL in first response who present high-risk features (*e.g.*, *TP53* deletions and/or mutations), and in those with early relapse after treatment with chemoim-

munotherapy, before tumor resistance becomes apparent. Yet, patients with CLL should not be denied transplantation just because of persistent, active disease. Patients with high-risk CLL should be managed in centers with expertise in CLL and hematopoietic stem cell transplantation and, whenever possible, be included in well-designed, large clinical trials. New agents that target specific disease pathways (*e.g.*, BCR kinase inhibitors, BCL-2 inhibitors, CDK inhibitors) are extremely promising since they are effective in refractory disease and in reducing bulky lymphadenopathy. Finally, a better understanding of graft *versus* CLL mechanisms is opening the door to new, immunomodulatory treatment strategies using T-cells, NK-cells, and dendritic cells. All these developments will hopefully make it possible to set up better and more effective treatment strategies for patients with high-risk CLL.

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