Bone Marrow Transplantation (2016) **51,** 799–806

www.nature.com/bmt

ORIGINAL ARTICLE

Efficacy of cisplatin-based immunochemotherapy plus alloSCT in high-risk chronic lymphocytic leukemia: final results of a prospective multicenter phase 2 HOVON study

M van Gelder[1], MH van Oers[2], WG Alemayehu[3], MCJ Abrahamse-Testroote[4], JJ Cornelissen[4], ME Chamuleau[5], P Zachée[6], M Hoogendoorn[7], M Nijland[8], EJ Petersen[9], A Beeker[10], G-J Timmers[11], L Verdonck[12], M Westerman[13], O de Weerdt[14] and AP Kater[2]

Allogeneic stem cell transplantation (alloSCT) remains the only curative option for CLL patients. Whereas active disease at the time of alloSCT predicts poor outcome, no standard remission-induction regimen exists. We prospectively assessed outcome after cisplatin-containing immune-chemotherapy (R-DHAP) followed by alloSCT in 46 patients (median age 58 years) fulfilling modified European Society for Blood and Marrow Transplantation (EBMT) CLL Transplant Consensus criteria being refractory to or relapsed (R/R) <1 year after fludarabine or <2 years after fludarabine-based immunochemotherapy or R/R with del(17p). Twenty-nine patients received $\geqslant 3$ cycles of R-DHAP and sixteen <3 cycles (4 because of disease progression, 8 for toxicity and 4 toxic deaths). Overall rate of response to R-DHAP was 58%, 31 (67%) proceeded to alloSCT after conditioning with fludarabine and 2 Gy TBI. Twenty (65%) remained free from progression at 2 years after alloSCT, including 17 without minimal residual disease. Intention-to-treat 2-year PFS and overall survival of the 46 patients were 42 and 51% (35.5 months median follow-up); del(17p) or fludarabine refractoriness had no impact. R-DHAP followed by alloSCT is a reasonable treatment to be considered for high-risk CLL patients without access or resistance to targeted therapies.

Bone Marrow Transplantation (2016) **51,** 799–806; doi:10.1038/bmt.2016.9; published online 15 February 2016

INTRODUCTION

Allogeneic stem cell transplantation (alloSCT) is the only potentially curative treatment for patients with CLL.[1–6] Evidence for a GvL effect comes from the observation that responses can occur after discontinuation of immunosuppression,[7–9] after donor lymphocyte infusion[10,11] and after onset of chronic GvHD (cGvHD).[7,12–14] AlloSCT overcomes the poor prognostic impact of fludarabine refractoriness and the presence of deletion(17p)[del (17p)],[1–3,8,15,16] and the GvL effect seems to be preserved after reduced intensity conditioning.[1,8,15,16] Therefore, alloSCT was proposed for patients with poor survival with non-transplant treatments: (1) purine analog refractory/relapsing (R/R) <12 months, or (2) R/R <2 years after purine-analog-based combination therapy or (3) del(17p)/ TP53 abnormalities present (EBMT Transplant Consensus on alloSCT in CLL).[17]

High disease burden and chemorefractory disease at the time of alloSCT have been identified as negative predictors of PFS.[1,18,19] Therefore, before alloSCT effective remission-induction treatment is mandatory, but as of today no standard regimen exists. It is also currently unknown what percentage of patients fulfilling one or more of the EBMT criteria and planned for alloSCT, actually are being transplanted.

Here we report results of a prospective multicenter single-arm phase 2 study in high-risk CLL patients fulfilling one or more of the EBMT Transplant Consensus criteria, where a uniform remission-induction therapy with R-DHAP (cisplatin, cytarabine, steroids and rituximab) before alloSCT was applied. The rationale for choosing R-DHAP was derived from both clinical observations and *in vitro* studies. DHAP induced a response in 8/10 patients with fludarabine-refractory or relapsed disease,[20] and a similar response rate was suggested from a small retrospective study.[21] *In vitro* studies demonstrated that platinum-based compounds induce TP53-independent cell death of CLL cells from chemorefractory CLL patients.[22,23] Rituximab was added to the DHAP chemotherapy backbone as it appeared to be of additive value in the relapsed setting when combined with chemotherapy.[24,25]

PATIENTS AND METHODS

Patients

Eligible patients were in need of treatment[26] with poor-risk CLL according to one of the following modified EBMT Consensus criteria[17]: fludarabine refractoriness (defined as no response or relapse <12 months after the last administration of fludarabine), or relapsed <24 months after the last

[1]Department of Internal Medicine, Maastricht University Medical Center, Maastricht, The Netherlands; [2]Department of Hematology and Lymphoma and Myeloma Center, Academic Medical Center, Amsterdam, The Netherlands; [3]HOVON Data Center, Erasmus Medical Center, Rotterdam, The Netherlands; [4]Department of Hematology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands; [5]Department of Hematology, Free University Medical Center, Amsterdam, The Netherlands; [6]Department of Hematology, Ziekenhuis Netwerk Antwerpen Stuivenberg, Antwerpen, Belgium; [7]Department of Internal Medicine, Medical Center Leeuwarden, Leeuwarden, The Netherlands; [8]Department of Hematology, University Medical Center Groningen, Groningen, The Netherlands; [9]Department of Hematology, University Medical Center Utrecht, Utrecht, The Netherlands; [10]Department of Internal Medicine, Spaarne Ziekenhuis, Hoofddorp, The Netherlands; [11]Department of Internal Medicine, Ziekenhuis Amstelland, Amstelveen, The Netherlands; [12]Department of Internal Medicine, Isala Klinieken, Zwolle, The Netherlands; [13]Department of Internal Medicine, Medisch Centrum Alkmaar, Alkmaar, The Netherlands and [14]Department of Internal Medicine, Antonius ziekenhuis Nieuwegein, Nieuwegein, The Netherlands. Correspondence: Dr M van Gelder, Department of Internal Medicine, Maastricht University Medical Center, Postbus 5800, 6202 AZ Maastricht, The Netherlands.
E-mail: m.van.gelder@mumc.nl
Received 10 November 2015; revised 22 December 2015; accepted 31 December 2015; published online 15 February 2016

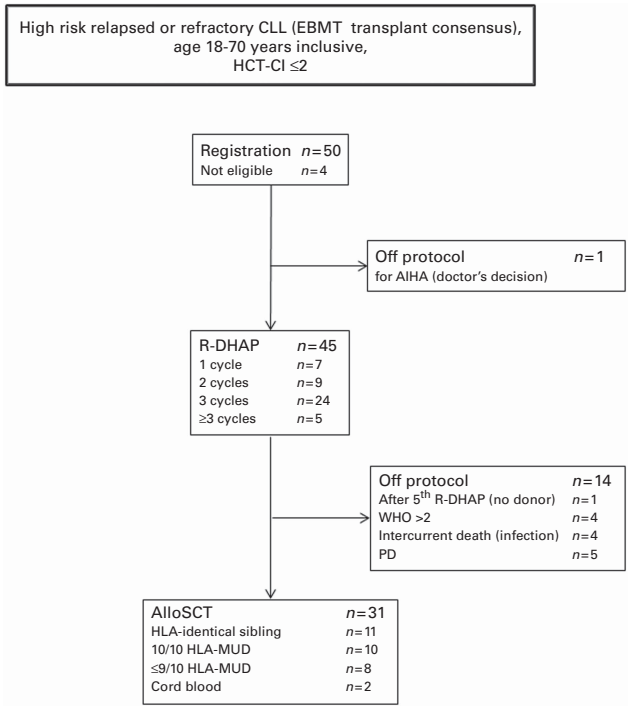

Figure 1. Flowchart for on protocol treatment and reasons for going off protocol of the 50 included patients. AIHA = auto-immune hemolytic anemia; alloSCT = allogeneic stem cell transplantation; HCT-CI = hematopoietic cell transplantation comorbidity index; MUD = matched unrelated donor; PD = progressive disease.

administration of fludarabine combined with a monoclonal antibody, or refractory or relapsed and having del(17p). Patients had to be 18–70 years inclusive with a WHO performance $\leqslant 2$ and a hematopoietic cell transplantation—comorbidity index (HCT-CI) $\leqslant 2$.[27] For the exclusion criteria see Supplementary Material.

A donor search had to be initiated immediately after registration. Eligible donors were siblings or unrelated volunteers who were HLA-identical by high-resolution HLA-A, -B, -C and -DR typing; 7/8 HLA-allele-matched donors were accepted. The study was approved by the Dutch and Belgian central and by all local ethical committees. Signed informed consent forms were obtained from all included patients before registration. During the inclusion period no competitive studies were open in the Netherlands and Belgian. Idelalisib or ibrutinib were not available at that time.

Treatment

Following registration, patients were scheduled for treatment with at least three R-DHAP cycles[28] (for dose reduction schedule see Supplementary Material). The dose of rituximab was 375 mg/m^2 in cycle 1 and 500 mg/m^2 in subsequent cycles. Non-progressive patients with a $\geqslant$7/8 HLA-allele matched donor and without uncontrolled infections and HCT-CI $\leqslant 2$[27] were allowed to proceed with alloSCT. Conditioning was with IV fludarabine (30 mg/m^2) for 3 days and 2.0 Gy TBI. GvHD prophylaxis was by cyclosporin and mycophenolate-mofetil or enteric-coated mycophenolate sodium.[1] Minimal residual disease (MRD) was measured by flowcytometry using a three tube system being effective at the 0.01% threshold.[29] Response evaluation was according modified National Cancer Institute criteria.[26]

Statistical considerations and methods

The primary end point of the study was 2-year PFS from registration with progression defined as progression or relapse[26] or death due to any cause whichever came first. The study aim was to assess the feasibility and efficacy of remission-induction chemotherapy followed by alloSCT. The sample size calculation was based on the assumptions that 2-year PFS of patients fitting the entry criteria was ~25% without alloSCT at the time this study started[17,24,30–35] and that protocol treatment would result in 2-year PFS of at least 45% from registration, which resulted in 41 required patients (for details see Supplementary Material). All analyses were intention to treat, restricted to eligible patients. The Mann–Whitney *U*-test was used to compare times between registration and alloSCT for recipients of stem cells from sibling and unrelated donors. PFS and overall survival (OS) probabilities were estimated applying the Kaplan–Meier method. To assess the feasibility of the study with regard to the primary end point, the PFS (from registration) at 2 years together with the 95% confidence interval (CI) was estimated. In the analysis of the PFS, the failure events (progression, death without progression) were considered as competing risk events. Univariate Cox-regression analyses were applied to test differences in PFS with regard to patient characteristics at entry (age, bulky lymphadenopathy ($\geqslant$1 lymph node with a diameter $\geqslant$5 cm on computed tomography (CT) scan)), del (17p); and at alloSCT (stable disease (SD) vs responsive, WHO performance, HCT-CI, EBMT scores). Hazard ratios (HR) and 95% CIs based on univariate Cox-regression analyses were estimated. Associations of selected risk factors were investigated applying logistic regression. All reported *P*-values are two-sided, and have not been adjusted for multiple testing.

Table 1. Characteristics of the 46 eligible patients

	Number (total N = 46)	*%*
Age (years)		
Median	58	
range	(43–69)	
male sex	35	76
WHO performance		
0	25	54
1	16	35
2	2	4
Unknown	3	7
del(17p)		
Present	18	39
Absent	28	61
EBMT risk (hierarchical)[38]		
del (17p)	18	39
Fludarabine refractory[a]	18	39
Fludarabine combination therapy refractory[a]	10	22
Bulky		
Present	20	43
Absent	22	48
Unknown	4	9
Number of prior therapies		
1	15	33
2	10	22
3	10	22
4	8	17
5	3	7

[a]According to the definitions as described in Patients and Methods section.

RESULTS

Patient characteristics at entry

Fifty patients were included between February 2009 and December 2012; four were ineligible (Richter's syndrome ($n = 2$), uncontrolled infection ($n = 1$) and a psychological condition hampering study protocol compliance ($n = 1$)) and were excluded from all analyses (Figure 1). Eighteen patients had del(17p) and 18 other patients had fludarabine-refractory disease. Other characteristics of the eligible patients are given in Table 1.

R-DHAP remission induction

Dosing. Twenty-nine patients received $\geqslant$3 R-DHAP cycles (63%; five had >3 cycles), 9 received two cycles (20%) and 7 received

Table 2. Efficacy of R-DHAP treatment in the 45 patients who were actually treated

	ORR (%)	*MRD − CR (%)*	*CR (%)*	*PR (%)*	*SD (%)*	*PD (%)*	*NPM (%)*	*Not known*[a] *(%)*	*Proceeding with alloSCT (%)*
All patients ($n=45$)	27 (60)	2 (4)	2 (4)	23 (51)	6 (13)	5 (11)	4 (9)	3 (7)	31 (69)
EBMT risk (hierarchical)[38]									
del (17p) ($n=17$)	10 (59)	1 (6)	2 (12)	7 (40)	3 (18)	1 (6)	3 (18)	—	12 (71)
Fludarabine refractory ($n=17$)	10 (59)	—	—	10 (59)	1 (6)	2 (11)	1 (6)	3 (18)	10 (59)
Fludarabine combination therapy refractory ($n=11$)	7 (63)	1 (9)	—	6 (55)	2 (18)	2 (18)	—	—	9 (81)
Bulky lymphadenopathy									
Yes ($n=19$)	13 (68)	1 (5)	2 (11)	10 (53)	—	4 (21)	1 (5)	1 (5)	12 (63)
No ($n=22$)	14 (64)	1 (5)	—	13 (59)	6 (27)	—	—	2 (9)	19 (86)
Unknown ($n=4$)		—	—	—		1 (22)	3 (5)	—	0 (88)

Abbreviations: alloSCT = allogeneic stem cell transplantation; MRD = minimal residual disease; NPM = non-progression-related mortality; ORR = overall response rate, PD = progressive disease; SD = stable disease. [a]Due to toxicity no response measured.

only 1 (17%; Figure 1). The vast majority received full doses (Supplementary Table 1). Four had cisplatin dose reductions because of decreased renal function. Reasons for not receiving 3 R-DHAP cycles were toxicity (eight patients, of whom three had a documented response at the time of discontinuation), non-progression-related mortality (NPM, four patients) and progressive disease (PD) (four patients, three after the 1st cycle of R-DHAP, one after the 3rd). One patient never received R-DHAP (doctor's decision).

Efficacy. Overall response rate after R-DHAP was 58% (95% CI: 43–73%): 2 MRD-negative CR (4%), 2 CR (4%) and 23 PR (50%; Table 2). Six patients had stable disease (13%) and five PD (11%). Response could not be assessed in 8 (18%) because of early death or going off protocol because of toxicity. Response rates did not significantly differ between patients with a different transplant indication according to EBMT Transplant Consensus criteria (odds ratios (OR) and 95% CI were 1.10(0.28–4.26) for fludarabine refractoriness versus del(17p) and 1.05(0.21–5.16) fludarabine combination therapy refractory versus del(17p) respectively; overall $P=0.99$), or between patients with or without bulky lymphadenopathy (OR = 1.06 (0.30–3.76); $P=0.93$; Table 2).

Toxicity. Forty-four grade $\geqslant 3$ non-hematologic adverse events were observed in 36 patients (Supplementary Table 2). Among the first 16 patients 4 in whom bacterial prophylaxis had not been given developed sepsis (three died). After centers had been instructed to strictly adhere to anti-bacterial prophylaxis (in most cases fluoroquinolones) no lethal infections were observed in the subsequent 30 patients. Total NPM during R-DHAP remission induction was 9% (4/45). One patient had tumorlysis after the first R-DHAP cycle. Renal function was not impaired after the last R-DHAP cycle in almost all patients; only four patients had grade 1 renal toxicity. The reasons for going off protocol of the four patients that were not assessable for response evaluation were poor performance due to infections ($n=3$) and cisplatin-induced seizures.

AlloSCT

Thirty-one patients proceeded to alloSCT (67%; Figure 1), of whom 27 had received $\geqslant 3$ R-DHAP cycles and four patients 2 cycles. Of the 12 transplanted patients who had bulky lymphadenopathy at start of R-DHAP treatment, only 2 had still bulky lymphadenopathy at the time of alloSCT. Appropriate donors were found for 29 patients and in all but one G-CSF-mobilized peripheral blood stem cells (PBSC) were used as stem cell source. ATG was added to the conditioning regimen in ten patients (all 18 matched unrelated donor (MUD) recipients); four of these had a $<10/10$ HLA-matched donor. Two patients received cord blood stem cells. These and other disease- and transplant-related characteristics are listed in Table 3. The median time from registration to alloSCT for patients transplanted from sibling donors was 111 days (mean 122, range 90–167), and 134 days for recipients of MUD donor stem cells (mean 162, range 88–510, $P=0.07$). Twenty-three patients achieved full donor chimerism, three had between 85% and 95% documented donor chimerism, one had maximal 82% donor chimerism and two died from aGvHD before being tested for chimerism (Figure 2). In two other patients chimerism was not tested. One patient rejected the donor graft, but full donor engraftment resulted after a second transplant from the same donor.

Efficacy. Following alloSCT, the response status improved in 19 of 24 evaluable patients (Table 4; Figure 2). Eighteen achieved MRD-negative CR (75%; 2 patients had no formal CR because a CT-scan had not been performed), 2 CR (MRD not tested) and 1 PR at any time point after alloSCT. Two had SD, which persisted in one despite administration of three escalating doses of donor lymphocyte infusion. Five patients had PD after transplantation (Figures 2, 3a) of whom one more than 4 years after transplantation; four died and one was alive at last follow-up with ibrutinib responsive disease.

Documented MRD negativity at any time after alloSCT occurred in 4 patients during tapering of immunosuppression, in 12 soon after cessation of immunosuppression and in 2 at later time points. All but one MRD-negative patients had cGvHD.

PFS at 1 and 2 years after alloSCT were 65% (95% CI 45–79%) and 61% (95% CI 42–76%; Figure 3c), median PFS was 50 months (95% CI 8 months—not reached) with a median follow-up of patients alive of 32 months (range 15–59). In univariate analysis only age had a statistically significant impact on PFS (HR 1.09, 95% CI 1.01–1.17, $P=0.029$), whereas number of prior therapies (as a continuous variable), del(17p), fludarabine refractoriness, R-DHAP responsive disease, donor type (HLA-identical sibling, 10/10 HLA-matched and $<10/10$ HLA matched), EBMT score, WHO score (0 or $\geqslant 1$) and HCT-score (0 vs $\geqslant 1$) at the time of alloSCT had not.

Toxicity. Grades 2–4 aGvHD occurred in 14 patients (45%); 4 had a sibling donor, 9 had a MUD and one received cord blood stem cells. aGvHD was lethal in six (1 had a sibling donor, 4 had a MUD (2 10/10 and 2 9/10 HLA-matched (the latter had received ATG as part of the conditioning)) and 1 had received cord blood). One other patient died from leukoencephalopathy most likely calcineurin inhibition-related as infections like JC virus, CMV and

Table 3. Characteristics of the 31 transplanted patients

	Number (total N = *31)*	*%*
Age, median	57	
Range	43–69	
Disease status at alloSCT		
CR, MRD −	1	3
CR	2	6
PR	22	71
SD	6	19
EBMT risk (hierarchical)[38]		
del (17p)	12	39
Fludarabine refractory	11	35
Fludarabine combination therapy refractory	8	26
Bulky lymphadenopathy at alloSCT	2	7
WHO performance		
0	19	61
1	12	39
HCT-CI		
0	15	48
1	9	29
2	2	6
3	2	6
>3	3	13
Donor		
HLA-identical sibling	11	36
10/10 HLA-matched unrelated	10	32
7/8 HLA-matched unrelated	8	26
Cord blood	2	6
Female donor for male patient		
Yes	9	29
No	22	71
CMV status patient		
Positive	16	52
Negative	14	45
Unknown	1	3
'Modified' EBMT score		
3	3	10
4	11	35
5	13	42
6	4	13

Abbreviations: alloSCT = allogeneic stem cell transplantation; HCT-CI = hematopoietic cell transplantation—comorbidity index; MRD = minimal residual disease; SD = stable disease; WHO = World Health Organization.

toxoplasmosis were excluded. Nineteen of the 26 patients at risk for cGvHD developed cGvHD (Figure 2) and two of them died as a result of cGvHD. NPM was 16% at 100 days post alloSCT and 26% at 2 years (Figure 3b).

Intention-to-treat analysis (from study entry)

At closure of the database (12 January 2015) 18 of the 46 eligible patients were still alive with a median follow-up of 35.5 months (range 0.2–62); 17 of the 18 patients still alive had been transplanted on protocol.

The overall response rate (ORR) was 67% (95% CI 53–82%). The primary end point PFS at 2 years was 42% (95% CI 27–56%) (Figure 4a) and the median PFS was 16 months (95% CI 8–54 months). Twelve patients died from PD (26%) and 16 (35%) from NPM.

In univariate analysis, age had a statistically significant influence on PFS (HR 1.07, 95% CI 1.0–1.1, $P = 0.009$). There was a trend for bulky lymphadenopathy at the time of starting R-DHAP to predict shorter PFS (HR 2.04, 95% CI 0.9–4.5, $P = 0.08$). The reason for this trend is unclear because of the heterogeneous nature of the events: sepsis during R-DHAP, or disease progression either during R-DHAP or early or late after alloSCT, and lethal aGvHD. A longer time between study entry and alloSCT (median time 4.1 months, range 3.0–8.2) also resulted in a shorter PFS (HR 1.63, 95% CI 1.03–2.57, $P = 0.053$). The presence of del(17p) (HR 0.44, 95% CI 0.3–1.6, $P = 0.44$) or fludarabine refractoriness (HR 1.23, 95% CI 0.59–2.58, $P = 0.59$) had no impact.

According to the ITT analysis, the 2-year OS was 51% (95% CI 36–64%) and the median OS 24 months (95% CI 9 months—not reached; Figure 4b).

DISCUSSION

AlloSCT is currently the only potentially curative treatment for high-risk CLL.[1–6] The drawbacks of this procedure are NPM and cGvHD-related morbidity, justifying alloSCT only for very high-risk CLL patients.[17] Our report is the first that prospectively describes outcome of patients that fulfill one or more of the EBMT Transplant Consensus criteria, have a treatment indication and entered the study at the start of remission induction with the aim to proceed to alloSCT. Because of the urgent treatment indication and because as of today no established effective salvage remission-induction treatment before alloSCT is available, we chose R-DHAP based on promising clinical and *in vitro* data.[20,23,36,37] R-DHAP resulted in remission or SD in the majority of patients of whom almost all subsequently proceeded to alloSCT (patients with progressive disease were not transplanted according to the protocol). The total treatment sequence of R-DHAP and alloSCT (only for those with at least stable disease after R-DHAP, a suitable medical and physical condition and the availability of a donor) resulted in a 2-year PFS of 42% (95% CI 27–56%), while based on literature before the use of targeted agents, a 2-year PFS of 25% was expected without alloSCT. This indicates that patients fitting the entry criteria of this study may fare better when treated with alloSCT than with non-alloSCT (immune-)chemotherapy based treatments, which is in line with a recent retrospective donor vs no donor comparison.[38]

The 58% ORR to R-DHAP compares favorably with recent publications on the use of platinum and cytarabine in high-risk CLL,[20,36,37] and we confirmed that the response rate is independent of the presence of del(17p). Infections were common during R-DHAP remission induction, but it must be emphasized that strict adherence to the use of bacterial prophylaxis as was mandated from the 16th patient prevented the occurrence of severe sepsis. Eight patients received 2 instead of 3 cycles of R-DHAP due to toxicity, although 4 nonetheless could proceed to alloSCT. Renal toxicity was infrequent and, when occurring, reversible by IV saline infusion in almost all cases, and cisplatin dose reduction for renal toxicity was rarely required. We feel that when bacterial prophylaxis is standardly applied, R-DHAP is an effective and feasible bridging therapy in high-risk CLL patients scheduled for alloSCT.

The results of alloSCT in this study with respect to NPM were slightly inferior to some retrospective reports of T-replete alloSCT[3,19] but not all,[1] and do not differ from EBMT registry based data,[39–41] while cumulative incidence of relapse and 2-year PFS seem slightly superior to the EBMT registry data and similar to the other reports. The cumulative incidence of MRD-negative CR is similar as reported in the retrospective studies.[1,3,19] This indicates that remission induction by R-DHAP did not compromise the anti-CLL effect of the successively performed alloSCT.