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Elimination of minimal residual disease in chronic lymphocytic leukemia using allogeneic stem cell transplantation

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A B S T R A C T

The kinetics of minimal residual disease (MRD) in peripheral blood were prospectively measured using real-time immunoglobulin heavy chain polymerase chain reaction (PCR) in nine patients with unmutated chronic lymphocytic leukemia (CLL) after non-myeloablative allogeneic stem cell transplantation (allo-NST). NST conditioning provided only a moderate reduction in median MRD levels ($5.4x10^{-2}$ pretransplant vs. $5.0x10^{-3}$ at +3 months). However, after withdrawal of immunosuppression, MRD levels progressively declined to $5.0x10^{-5}$ at +5 months and to MRD negativity at +12 months in seven of nine patients. With a median follow-up of 40 months (range 31–53 months), six of these seven patients remained in continuing clinical and molecular remission. In one patient, however, CLL relapsed as high-grade gastric lymphoma 3 years post-allo-NST despite long-term and ongoing MRD negativity in the peripheral blood. These results, taken together, show for the first time that a progressive decline in MRD levels to negativity can be obtained following NST for unmutated CLL, suggesting a crucial role for graft-versus-leukemia activity-mediated immunotherapy in complete disease eradication in this subset of high-risk patients.

Allogeneic stem cell transplantation (allo-SCT) has been shown to result in long-term disease control in a proportion of patients with resistant chronic lymphocytic leukemia (CLL),[1] and there is some evidence that graft-versus-leukemia (GVL) activity is of crucial importance for the efficacy of allo-SCT.[2-4] To date, however, it is unknown whether GVL can be effective in patients with unmutated V_H status and an unfavorable karyotype who have a poor prognosis following conventional chemotherapy.[5] Information on this issue is particularly important as allo-SCT in CLL is being increasingly performed using non-myeloablative or reduced-intensity conditioning,[6-8] which implies that the contribution of GVL to disease control will become even more essential. Thus, we aimed to investigate whether the therapeutic resistance of unmutated CLL could be overcome by allo-SCT following non-myeloablative conditioning (NST), and to compare the relative contributions of conditioning regimen and GVL to tumor control. As the preliminary results of this study have already been published,[9] the present report focuses on follow-up and complementary information.

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Design and Methods

Patients included in this study were consecutive patients from an ongoing prospective trial on NST in CLL[10] who had an unmutated immunoglobulin heavy-chain (IgH) gene variable region (VH) and a clone-specific PCR primer with a sensitivity level of at least 10^{-4}, as well as diagnostic material available for longitudinal quantitative molecular monitoring. Allogeneic peripheral blood stem cell grafts were harvested from an HLA-identical sibling or unrelated donors after stimulation with granulocyte colony-stimulating factor. Conditioning consisted of daily fludarabine (30 mg/m^2) and cyclophosphamide (500 mg/m^2) over 5 days. Graft-versus-host disease (GVHD) prophylaxis was performed using cyclosporine A (CSA) and short-course methotrexate. In the case of unrelated donors, antilymphocyte globulin was added. CSA was tapered between day +60 and +100 post-transplantation in the absence of GVHD. Donor lymphocyte infusions (DLI) were administered from day +120 onwards in the case of incomplete chimerism or residual disease following CSA withdrawal. The protocols were approved by institutional review boards. IgH sequencing, mutational analysis, quantitative CDR3 PCR and chimerism assessment were performed as described elsewhere.[9] Two-tailed non-parametric Mann-Whitney tests and Fisher's exact test were used to compare quantitative and qualitative parameters, respectively, between subgroups of patients.

Table 1. Clinical course of patients prior to allogeneic PBSCT.

UPN	sex/age (years)	% VH homology	FISH karyotype	LDT <12 mos.	Time from dx. (mos)	Max. Binet stage	Symptoms	Previous therapy	Previous auto-SCT	Allo-SCT indication	Status at allo-SCT
41P01	F/49	99.6	no abnormality	no	91	B	bulky disease	PmM x 3; FE x 5;DB x 2	no	H-RD	PR2
41P02	M/57	100	no abnormality	na	51	B	Richter's transform	Clb x 7; Flud x 3; DB x 1	no	Richter's transform; Flud failure	PR2
05P04	M/53	100	del 11q22-q23, del 13q14	yes	44	B	bulky disease; B-symptoms	CHOP x 4;DB x 1; FC x 4; CY x 1	no	H-RD	UTR
41P03	M/40	100		na	8	B	bulky disease	FC x 3	no	H-RD	PR1
05001	M/48	100	del 11q22-q23	yes	129	C	bulky disease; anemia; thrombocytopenia	Clb x 20; Trofo x 1; CHOP x 1; DB x 2; auto-SCT; FC x 4	yes	H-RD; auto-SCT failure	PR3
05002	M/43	100	del 13q14, t(14q32)	yes	44	A	B-symptoms; LDT <3 mos.	CHOP x 3; DB x 1; auto-SCT; FC x 1	yes	H-RD; auto-SCT failure	PR2
05003	F/57	100	del 13q14	yes	57	B	bulky disease	Clb x 12; Flud x 5; FC x 2; DB x 1	no	H-RD	PR2
05004	M/63	100	del 17p13	no	43	B	B-symptoms; bulky disease	FC x 2; CHOP x 3; DB x 1; Rituximab x 4	no	H-RD; Flud failure	RD
41001	M/59	100	del 13q14	yes	18	B	bulky disease; WBC >400/mL	FC x 3; DB x 1	no	H-RD	PR1

H-RD, high-risk disease; PR: partial remission; UTR, untreated relapse; RD, refractory disease; BVD, adriamycin (ADR), bleomycin, vinblastin, dacarbazin; ASHAP, ADR, methylprednisolone, cytarabine (ARA-C), cisplatin (cDDP); CHOP: cyclophosphamide (CY), doxorubicin, vincristine, prednisolone; Clb: Chlorambucil; COPP, cyclophosphamide (CY), vincristine (VCR), procarbazine (PROC), prednisolone (PDN); D-B (Dexa-BEAM): dexamethasone, carmustin, etoposide, cytarabine, melphalan; DHAP, Dexa, ARA-C, carboplatin; F: female; FC: fludarabine, CY; Flud: fludarabine; IEP, ifosfamide, VP-16, PDN; M, male; MC, mixed cellularity; MIFAP, mitoxantrone, FLU, ARA-C, cDDP; NS, nodular sclerosis; OEPA, VCR, VP-16, PDN, ADR; OPPA, VCR, PROC, PDN, ADR; Trofo: trofosfamide.

Table 2. Conditioning regimen, graft characteristics and outcome following allogeneic PBSCT.

UPN	Conditoning regimen (dose in mg/kg or mg/m^2)	Donor (mos.)	CSA	GvHD Acute	GvHD Chronic	Time to chronic GvHD (mos.)	DLI (mos.)	BR	Time to BR	BC	Time to BC (mos.)	Status
41P01	Flu150/Cy2.5	Sibling	0–3	0	no	-	+6	CCR	-	100%	+4	alive 49+ mos. (rel. +44 mos)
41P02	Flu150/Cy2.5	Sibling	0–3	0	no	-	+6	MCR	+5	100%	+8	alive 53+ mos. (MCR)
05P04	Flu150/Cy2.5 /ATG	MUD	0–34+	II	ext	+4	no	MCR	+8	100%	+5	alive 49+ mos. (MCR)
41P03	Flu150/Cy2.5	Sibling	0–2	I (DLI)	no	no	+3 +4	MCR	+5	66%	+9	alive 41+ mos. (MCR)
05001	Flu150/Cy2.5	Sibling	0–3; 7–13	0	ext	+6	no	MCR	+8	100%	+5	alive 38+ mos. (MCR)
05002	Flu150/Cy2.5	Sibling	0–20	I	ext	+4	no	MCR	+7	100%	+2	alive 39+ mos. (MCR, but rel.+33 mos; see text)
05003	Flu150/Cy2.5	Sibling	0–22+	I	ext	+4	no	MCR	+5	100%	+5	alive 38+ mos.(MCR)
05004	Flu150/Cy2.5	Sibling	0–3; 6–?	I	ext	+4	no	CCR	+2	100%	+3	died +19 mos. (CLL)
41001	Flu150/Cy2.5	Sibling	0–4	III	ext	+8	no	MCR	+12	100%	+4	alive 31+ mos (MCR)

BR, best response; BC, best chimerism; ATG, antithymocyte globuline; CCR, clinical complete remission (CR); MCR molecular CR CSA, cyclosporine A; Cy, cyclophosphamide; DLI, donor lymphocyte infusions; Flu, fludarabine; MUD, matched unrelated donor.

Results

Patients

Altogether, nine patients with unmutated high-risk CLL who had received an allo-SCT after non-myeloablative conditioning met the inclusion criteria for this study. The median age at SCT was 53 years (40-63 years). The median time from diagnosis to transplant was 44 months (8-129 months), and patients had received 4 (range 1-6) prior chemotherapy regimens, including autologous SCT in two cases. Detailed pre-transplant characteristics are given in Table 1.

Kinetics of MRD

Following NST, the kinetics of clone-specific DNA copies in peripheral blood, as assessed by real-time PCR (RQ-PCR), basically showed a biphasic pattern with only a modest decrease in the early post-transplant phase, followed by a marked and sustained reduction in minimal residual disease (MRD) levels from day +100 onwards. Median MRD levels were reduced by approximately 1 log between the time prior to SCT and day +100 [$5.4x10^{-2}$ (range $2.9x10^{-3}$–$2.7x10^{-1}$) vs. $5.0x10^{-3}$ (range $2.8x10^{-3}$-$8.5x10^{-2}$), p=0.05] but by more than 2 logs between day +100 and day +200 [$4.0x10^{-5}$ (range $1.0x10^{-5}$-$9.9x10^{-3}$), p=0.006]. During subsequent follow-up, MRD levels stayed low and became undetectable in seven of nine patients (78%).

In five of the nine study patients (UPN 05P04, 05001, 05002, 05003, 41001), progressive disappearance of RQ-PCR-measurable MRD occurred subsequent to the development of extensive chronic GVHD. All of these five patients achieved ongoing MRD negativity. Patients UPN 41P01, 41P02, and 41P03 remained free of GVHD. Although DLI resulted in durable eradication of MRD without any GVHD in patients UPN 41P02 and 41P03 (in the latter without conversion to complete chimerism), elimination of MRD was not achieved in patient UPN 41P01. This patient had a clinical relapse 44 months post-transplant and is currently receiving salvage chemotherapy. Patient UPN 05004, who was the only carrier of a 17p13 deletion in this series, showed evidence of early clinical relapse prior to the onset of extensive chronic GVHD 4 months after NST. Although significant clinical remission of bulky lymphadenopathy did not occur, GVHD was associated with declining, albeit steadily, positive MRD levels until the patient died from complications of progressive disease at month +19.

Notably, in one of the MRD-negative patients (UPN 05002), CLL relapsed as high-grade gastric lymphoma 33 months post-allo-SCT despite ongoing MRD negativity in the peripheral blood. This patient is now in complete remission at +39 months after having received salvage chemotherapy. The remaining six patients are in ongoing complete clinical remission with a median follow-up of 40 months (31–53 months) post-transplant. Transplant-related deaths did not occur (Table 2).

Discussion

There is ample circumstantial evidence that GVL mechanisms contribute to the disease control that can be achieved by allogeneic SCT in CLL. This evidence includes the observation that late relapses rarely occur after allo-SCT,[4] findings that long-term molecular responses can be obtained with allo-SCT but not with auto-SCT,[3,4,11] a reduced relapse risk in the presence of chronic GVHD,[6] and anecdotal reports on the efficacy of DLI.[2,7] Direct proof of the existence and the immunotherapeutic capacity of the GVL principle in CLL, however, requires evidence of dynamic tumor response that is temporally correlated to immune interventions or GVHD in the absence of effective cytostatic treatment. These requirements have largely not been met in studies published to date since such studies were performed using myeloablative conditioning, lacked evidence of a clear-cut correlation between immune manipulation/GVHD and response and, most importantly, did not use methodologies suitable for longitudinal quantification of the tumor load at a subclinical level. In contrast, the present study was performed with a conditioning regimen that is unequivocally non-myeloablative and, for the first time, used RQ-PCR for highly sensitive MRD quantification, enabling a precise assessment of the tumor cell kinetics in response to immune interventions. Our results show that profound and sustained complete molecular responses occur only after establishment of chronic GVHD or DLI, whereas the influence of the conditioning regimen on the tumor cell load is very limited. The dynamic pattern of this process and its close correlation with immune-modulating maneuvers or chronic GVHD strongly suggest that GVL activity is responsible for tumor disappearance. On the other hand, the moderate cytoreduction provided by fludarabine/cyclophosphamide conditioning seems to be sufficient to allow the induction of effective GVL activity, at least in patients still responsive to fludarabine-based chemotherapy. Of note, all patients studied had an unmutated VH gene status, showing for the first time that genetically unfavorable unmutated CLL is sensitive to the GVL effect. Since the GVL-mediated antileukemic activity observed in this study is clearly superior to that of the intensified fludarabine/cyclophosphamide regimen used for conditioning, allo-SCT appears to be the most effective and, as yet, the only potentially curative modality available for the treatment of unmutated CLL. In contrast, autologous SCT can reduce but not eradicate PCR-measurable tumor load in the majority of patients with unmutated CLL.[9,12,13] Collectively, these findings show that GVL is working in unmutated CLL. The GVL-mediated antileukemic activity is sufficient to allow durable

complete elimination of measurable MRD without relevant contribution from the conditioning regimen, providing a rational basis for prospective studies on NST in CLL. MRD negativity is more often induced after allo-SCT than after autologous SCT, and is generally durable. This might be the result of complete disease eradication, or just an indicator of permanent disease control by ongoing GVL activity. That the latter scenario is possible is illustrated by the patient whose CLL relapsed as high-grade gastric lymphoma despite ongoing MRD negativity and the absence of typical clinical CLL manifestations. This constellation might be interpreted as ongoing suppression of the original *indolent* CLL clone by GVL, which is not, however, sufficient to control the transformed subclone representing the gastric lymphoma.

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