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EDITORIAL

The prognostic impact of minimal residual disease assessment after stem cell transplantation for chronic lymphocytic leukemia: is achievement of molecular remission worthwhile?

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For many decades, chronic lymphocytic leukemia (CLL) has been an incurable disease. With traditional therapies, mainly based on alkylating agents, the goal of treatment was clearly palliative. Although overall response rates of first-line therapy with alkylators ranged from 40 to over 70%, only a small fraction of patients reached complete remission, and the duration of responses was generally limited.[1–3] With this kind of treatment, quality of response could easily be assessed with conventional clinical and histopathological methods.[4]

Newer modalities, such as purine analogues, monoclonal antibodies, and stem cell transplantation (SCT) are, however, much more effective, resulting in longer remission duration and a relevant proportion of patients achieving complete remission.[2,3,5–10] The better quality of remission provided by these novel agents requires more sensitive tools for precise remission assessment. Methods capable of detecting and measuring CLL cells on a subclinical level (1) are needed for *quantification* of clinical complete remission, (2) could help to define the quality of remission as *prognostic marker*, and (3) may serve as *surrogate indicator of complete disease eradication* and cure, respectively.

Ideally, an assay for measurement of minimal residual CLL disease (MRD) should be CLL-specific, highly sensitive even in the presence of a majority of physiological B cells, broadly applicable, and capable of quantification. During recent years, two main approaches of MRD assessment in CLL have been followed: flow cytometry, taking advantage of the unique immunophenotype of CLL, and PCR-based strategies using the clonal rearrangement of the hypervariable region of the V_H part of the immunoglobulin heavy-chain gene (CDRIII region).[11–15] Both approaches have been refined now to allow specific detection and reliable quantification of residual CLL cells beyond one in 10^4, but allele-specific CDRIII PCR still seems to be the most sensitive assay.[13] It has to be kept in mind, however, that sensitivity is largely dependent on the sample size, that is to detect 1 in 10^4 cells, the total cell count in the sample should not be less than 1×10^5 for allele-specific CDRIII PCR and 2×10^6 for 4-color flow cytometry (4C-Flow).

SCT was the first treatment modality largely exceeding the efficacy of conventional therapy, thereby implementing the need for sensitive tools for response control. In particular, the hope that transplantation might be a curative treatment for CLL made MRD detection mandatory as a surrogate marker for disease eradication. On the other hand, SCT is a good model to illustrate that the predictive value of MRD assessment is strongly dependent on the treatment modality actually used, ie MRD negativity after autologous SCT (auto-SCT) has a prognostic meaning different from that after allogeneic SCT (allo-SCT). Moreover, lessons learned from SCT provide evidence that *MRD kinetics* are much more important than absolute MRD levels.

MRD measurement in CLL was first introduced by Gribben and coworkers in the context of the Dana-Farber Cancer Institute CLL transplant program. This group used a PCR methodology based on a FRI or CDRIII consensus primer CDRIII PCR. The PCR amplification product was blotted onto nylon membranes after agarose gel electrophoresis and visualized using 3′ end digoxigenin-labeled allele-specific oligonucleotides, which were generated after sequencing the patient-specific CDRIII region.[14] This assay was applied to blood and bone marrow (BM) samples obtained after auto-SCT and allo-SCT. Altogether, follow-up material was available for 40 patients who had undergone auto-SCT with B cell-depleted BM grafts after total body irradiation (TBI) and high-dose cyclophosphamide. Fifty-three percent of the patients achieved constantly or intermittently MRD negativity post-transplant and only one of these relapsed, whereas 53% of those remaining MRD positive subsequently relapsed (Donovan *et al. Blood* 1998; **92**: 652a; abstract) (Table 1). In addition, 16 patients were studied after allo-SCT. The high-dose regimen employed for conditioning was similar to that used in auto-SCT, and CD6-depleted BM grafts from HLA-identical siblings were transfused for hematopoietic reconstitution. MRD negativity was reached in 63% with only one subsequent relapse. In contrast, the relapse incidence in MRD-positive patients was 83% (Donovan *et al. Blood* 1998; **92**: 652a; abstract) (Table 2). During early follow-up, MRD reappearance after once having achieved MRD negativity was not observed either after auto-SCT or after allo-SCT.[14]

These results were confirmed by a study from the Kiel group who investigated MRD in 13 patients with CLL who had undergone a very similar high-dose regimen but received purged peripheral blood stem cells (PBSC) instead of BM for auto-SCT. MRD detection was performed with a less sensitive polyacrylamide gel electrophoresis-based consensus primer CDRIII PCR. MRD negativity was reached by 92% of the patients, and with a follow-up of 7–46 months, only one of them reconverted to positivity followed by clinical relapse.[16] Subsequent trials with longer follow-up and more sophisticated MRD-detection methodologies, however, yielded contrasting results (Table 1). The Barcelona group studied MRD by 3-color flow cytometry defining CLL cells by CD19, CD5 and CD20dim expression in 18 patients after auto-SCT. Myeloablation was

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Table 1 MRD studies in autologous SCT in CLL

	Dana-Farber (14)[a,b]	*Barcelona (17;18)*	*MRC (19)*	*GCLLSG*[c,d]
Study type	Single center retrospective	Single center retrospective	Multicenter prospective	Multicenter prospective
Method	Consensus PCR, ASO blot	3C-Flow (CD19, 5, 20dim)	Consensus PCR, (Genescan)	ASO RQ-PCR (Taqman)
Sensitivity	10-E3 to 10-E5	10-E4	10-E3 to 10-E4	10-E4 to 10-E5
Eligibility	Advanced/relapsed, chemosensitive, minimal disease,	Advanced, chemosensitive	Indication for first-line treatment	Indication for first-line treatment
Regimen	TBI/CY, purged BM	TBI/CY, unpurged PBSC	TBI/CY, purged/unpurged PBSC	TBI/CY, purged PBSC
n (total)	81	18	65	98
n (with marker)	52 (64%)	18 (100%)	43 (66%)	46 (47%)
MRD negative post-SCT	53%	61%	62%	56% (<10-E4)
MRD log reduction	NA	NA	NA	1.6
Prognostic	Pos: 53% relapsed	Pos: 86% relapsed	Pos: 63%	>10-E4: 36% relap
Impact of MRD	Neg: 5% relapsed	Neg: 9% relapsed	Neg: 17%	<10-E4: 0% relap
Continuous MRD increase/ reappearance	NA	Yes	Yes	Yes
4-year DFS	63%	40%	58%	57%
4-year OS	85%	NA	86%	NA
Follow-up (months)	30	28 (9–20)	36	36 (3–77)

3C-Flow, 3-colour flow cytometry; ASO, allele-specific oligonucleotides; BM, bone marrow grafts; CY, high-dose cyclophosphamide; DFS, disease-free survival; MRD, minimal residual disease; OS, overall survival; PBSC, peripheral blood stem cell grafts; RQ-PCR, real-time CDRIII PCR with allele-specific primers; TBI, total body irradiation; NA, not applicable.
[a](Gribben *et al. Blood* 1998; **92**: 322a; abstract).
[b](Donovan *et al. Blood* 1998; **92**: 652a; abstract).
[c](Ritgen *et al. Blood* 2004; **104**: 45a; abstract).
[d](Dreger *et al. Blood* 2004; **104**: 45a; abstract).

Table 2 MRD studies in allogeneic SCT in CLL

	Dana-Farber (14)[a,b]	*Barcelona (17;18)*	*Huddinge (20)*	*GCLLSG (21)*
Study type	Single center retrospective	Single center retrospective	Single center retrospective	Multicenter prospective
Method	Consensus PCR,	3C-Flow	ASO PCR	ASO RQ-PCR
ASO blot	(CD19, 5, 20dim)	(PAGE)	(Taqman)	
Sensitivity	10-E3 to 10-E5	10-E4	10-E4	10-E4 to 10-E5
Eligibility	Advanced/relapsed, chemosensitive, minimal disease	Advanced, chemosensitive	Advanced/relapsed, chemosensitive	Unmutated V_H, poor-risk
Regimen	TBI/CY, TCD-BM	TBI/CY, BM or PBSC	TBI/CY, BM	FLU/CY, PBSC
n (total)	23	14	6	9
n (with marker)	16 (70%)	14 (100%)	6 (100%)	9 (100%)
MRD negative post SCT	63%	70%	50%	77% (truly neg.)
Time to MRD negativity	NA	3–12 months	1–37 months	4–11 months
MRD log reduction	NA	NA	NA	>3
Prognostic	Pos: 83% relapsed	Pos: 0% relapsed	Pos: 33%	Pos: 100% relap
Impact of MRD	Neg: 10% relapsed	Neg: 0% relapsed	Neg: 0%	Neg: 14% relap
Continuous MRD increase/ reappearance	NA	No	No	No
4-year DFS	44%	67% (EFS)	NA	51%
4-year OS	50%	NA	NA	89%
Follow-up (months)	30	43 (15–106)	36	40 (31–53)

3C-Flow, 3-colour flow cytometry; ASO, allele-specific oligonucleotides; BM, bone marrow grafts; CY, high-dose cyclophosphamide; DFS, disease-free survival; EFS, event-free survival; FLU, fludarabine; MRD, minimal residual disease; OS, overall survival; PBSC, peripheral blood stem cell grafts; RQ-PCR, real-time CDRIII PCR with allele-specific primers; TBI, total body irradiation; TCD, T cell depletion with anti-CD6 and complement; NA, not applicable.
[a](Gribben *et al. Blood* 1998; **92**: 322a; abstract).
[b](Donovan *et al. Blood* 1998; **92**: 652a; abstract).

again based on TBI/cyclophosphamide, and unpurged PBSC were used as autografts. Although only one out of 11 patients who achieved MRD negativity showed clinical relapse during the observation period, there was a continuous pattern of MRD reappearance.[17,18] MRD studies were also performed in the prospective multicenter trial on early transplant in CLL with need for treatment undertaken by the UK Medical Research Council (MRC). The high-dose regimen was similar to the

previous studies, and purged or unpurged PBSC grafts were allowed. MRD was assessed by Genescan-based consensus primer CDRIII PCR. Despite absence of detectable MRD in 63% of 29 cases tested at 6 to 12 months post transplant, MRD negativity was not durable with only 45% remaining so at 36 months post SCT.[19]

Finally, MRD results are available for a subset of patients treated in a very similar multicenter trial performed by the German CLL Study Group (GCLLSG) (Ritgen *et al. Blood* 2004; **104**: 45a; abstract). In 46 patients, quantitative MRD measurement was possible using a sensitive real-time CDRIII PCR with allele-specific primers (RQ-PCR). Auto-SCT resulted in a strong reduction of the CLL load (median MRD level 2E-2 pretransplant *vs* 8E-5 at 50–150 days post transplant; $P=0.0001$), with no significant difference between patients with mutated and unmutated variable part of the immunoglobulin heavy chain gene (V_H gene), respectively. True MRD negativity, however, was achieved only occasionally and was not durable. Whereas MRD levels had no prognostic impact during the first 6 months after SCT, stable or decreasing MRD kinetics below 1E-4 between 6 and 12 months after SCT were strongly predictive for a favorable outcome (4-year progression-free survival (PFS) 100%). In contrast, PFS of those patients who had increasing MRD levels at this time was significantly poorer (4-year PFS 37%; $P=0.02$; $n=25$). Patients with increasing MRD levels early post-transplant almost exclusively belonged to the subgroup with unmutated V_H. These results demonstrate that due to prognostically relevant features other than the MRD response, such as the mutational status of the tumor cells, MRD *kinetics* rather than the pure MRD status immediately post treatment give important prognostic information.

The relevance of *longitudinal* MRD determination is underlined by the results obtained with *allo-SCT*, which are fundamentally different from those observed after auto-SCT. This was first reported by the Barcelona group, who investigated 14 patients after allo-SCT in parallel to the 18 auto-SCT cases mentioned earlier. Patients were allografted with unmanipulated BM or PBSC from HLA-identical sibling donors after myeloablative conditioning with TBI/cyclophosphamide. MRD reappearance was not observed in any of six patients initially converted to negativity. Moreover, MRD disappeared during longer follow-up at least in one of four cases remaining MRD positive immediately post transplant[18] (Table 2). These results were also mirrored by a similar study from the Huddinge Group. They found delayed MRD clearance in 3 out of 6 patients after myeloablative allo-SCT using CDRIII PCR with allele-specific primers on polyacrylamide gel electrophoresis.[20]

Recently, our group measured the kinetics of MRD by RQ-PCR as described above in nine patients with unmutated CLL after nonmyeloablative allo-SCT.[21] Conditioning for allo-SCT with fludarabine and cyclophosphamide provided only a moderate reduction of the median MRD level (5.4E-2 pretransplant *vs* 5.0E-3 at $+3$ months). After withdrawal of immunosuppression, however, MRD levels progressively declined to 5.0E-5 at $+5$ months and to MRD negativity at $+12$ months in seven of nine patients. With a median follow-up of 40 (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-SCT despite long-term and ongoing MRD negativity in the peripheral blood (P Dreger and M Ritgen, unpublished observations). In conclusion, this study shows that a progressive decline of MRD levels to negativity can be obtained after *nonmyeloablative* allo-SCT even for *unmutated* CLL, suggesting a crucial role of GVL-mediated immunotherapy for complete disease eradication in this high-risk subset. The curative potential of GVL activity in unmutated CLL is also supported by the fact that long-term clinical remissions can be observed after allogeneic allo-SCT but not after auto-SCT.[21,22]

Taken together, in contrast to auto-SCT, MRD negativity even by highly sensitive methods is reached after allo-SCT more often, and is generally durable. This might reflect complete disease eradication, but at least is an indicator of permanent disease control by ongoing GVL activity. That the latter scenario is possible is highlighted by the course of our patient whose CLL relapsed as high-grade gastric lymphoma.

In conclusion, results obtained with SCT illustrate that the predictive value of MRD assessment is strongly dependent on the treatment modality actually used, i.e. MRD negativity after auto-SCT has a prognostic meaning different from that after allo-SCT. In general, *MRD kinetics* seem to be much more important for outcome prediction than absolute MRD levels. In auto-SCT, MRD kinetics is just one prognostic factor, which must be regarded in the context of others, whereas in allo-SCT durable MRD negativity may be a true indicator of cure. Therefore, achievement of MRD negativity after allo-SCT is clearly worthwhile. In auto-SCT, however, reaching MRD negativity *per se* does not seem to be mandatory to assure a superior prognosis unless more potent, potentially curative auto-SCT approaches have been developed.

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