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LETTER TO THE EDITOR

Different molecular levels of post-induction minimal residual disease may predict hematopoietic stem cell transplantation outcome in adult Philadelphia-negative acute lymphoblastic leukemia

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Minimal residual disease (MRD) is a powerful indicator of the risk of relapse in adult acute lymphoblastic leukemia (ALL),[1] used for the risk-oriented application of allogeneic stem cell transplantation (allo-SCT) in patients who remain MRD-positive (MRD+) following induction and consolidation chemotherapy.[2–4] Although allo-SCT is less effective in MRD+ state,[5–7] correlations between post-induction quantitative MRD ranges and SCT outcome have not been clearly defined. This would allow an early identification of MRD+ patients at higher risk of posttransplantation failure, for whom a closer MRD monitoring and other therapies could be recommended before and after allo-SCT. The quantitative MRD to SCT relationship is examined in the final update of a prospective Northern Italy Leukemia Group (NILG) trial. In this study, post-induction MRD positivity was the sole decisive factor for the allocation to allo-SCT of adult patients with Philadelphia chromosome-negative (Ph−) ALL.

NILG trial ALL 09/00 was conducted between 2000 and 2006 (Supplementary Figure S1). Details of molecular MRD analysis, risk classification and application of risk/MRD-oriented therapy in the first 280 patients (192 with Ph− ALL) were published.[2] For MRD analysis, one or two patient-specific molecular probe(s) were used, with a sensitivity of at least 10^{-4}, and the bone marrow was examined at weeks 10, 16 and 22, that is, after 3, 5 and 7 treatment blocks, respectively. Patients with MRD $\geqslant 10^{-4}$ at time point 2 (TP2, week 16) and/or with any detectable positivity at TP3 (week 22) constituted the MRD+ group and were eligible for allo-SCT from human leukocyte antigen-matched related or unrelated donors. To avoid treatment delay, the donor search was initiated at complete remission (CR). No specific conditioning regimen was recommended. MRD+ patients without donor received high-dose treatment ('hypercycles') with autologous stem cell rescue (auto-SCT), followed by maintenance. MRD-negative (MRD−) patients were to receive standard maintenance therapy. The only exception to this design was t(4;11)+ ALL, always eligible for allo-SCT.

The primary objective of the current analysis was to determine whether different post-induction MRD levels were predictive of posttransplantation outcome in MRD+ patients. To this end the highest quantitative MRD value from all three study TPs qualified individual patients for inclusion into a given MRD subset. Patients with all negative MRD determinations were assigned to the complete molecular remission (CMR) group. The remaining patients formed the molecularly responsive (MR) subset, with all MRD signals below 10^{-4}, and two molecularly resistant groups with one or more MRD determinations ranging from 10^{-4} to $<10^{-3}$ (MR_1) and $\geqslant 10^{-3}$ (MR_2). Survival, disease-free survival (DFS) and relapse incidence (RI) were compared by MRD category in unselected patients and in those allocated to SCT in keeping with study design. Kaplan–Meier graphs, the log-rank and two-tailed chi-squared tests were used as appropriate for data reporting and comparative analyses among patient groups.[2]

The study enrolled 304 patients with Ph− ALL (Table 1). Two-hundred fifty-eight entered CR (85%). Sensitive molecular probe(s) were available for 200 CR patients (77.5%). Of these, 141 completed consolidation (70.5%) and 59 did not because of early SCT ($n=13$), relapse ($n=41$) and treatment toxicity ($n=5$). One-hundred thirty-six of 141 evaluable patients completed the MRD study: 76 were classified MRD− (56%) and 60 MRD+ (44%) (Supplementary Figure S2). Forty-three of the 60 MRD+ patients (71.6%) underwent SCT as per protocol design (26 allo-SCT, 17 'hypercycles' with auto-SCT) after a median of 2.2 months from the last consolidation cycle (range 0.5–15.4 months). Allo-SCT was from unrelated and sibling donors in 14 and 12 patients; and the stem cell source was bone marrow in 11, peripheral blood in 13 and cord blood in 2 patients, respectively. Long-term study results are available in Supplementary Figure S3, including outcomes according to clinical risk class. According to the current analysis, there were 64 CMR patients (47%), 21 MR patients (15.5%), 17MR_1 patients (12.5%) and 34 MR_2 patients (25%). Notably, these were all distinct subjects, summing up to the total of 136 MRD-evaluable cases, with no overlapping across different MRD subgroups. Therefore, all CMR-negative patients were MRD− at all evaluable TPs, and as such were excluded from allo-SCT by design (Table 1). Apart from that, a proportion of the remaining patients could express lower MRD levels at some TP, a finding that was progressively less frequent from MR_1 to MR_2 patients (<10% CMR and 20% MR at another TP) and affected mainly different individuals, suggesting consistency of the MRD risk reclassification, as already indicated in this clinical study by the strong statistical correlation between MRD TP1 and TP2/3 results.[2] After a minimum observation of 4 years and a maximum close to 13.5 years, estimated 6-year survival and DFS rates ranged from 73% and 64% in CMR patients to 24% and 15% in MR_2 patients, respectively, mostly in relation with an increasing RI (Figures 1a–c, all *P*s <0.0001), except for CMR and MR groups. Although 6-year DFS was improved following allo-SCT in MRD+ patients (42% versus 18% with auto-SCT, $P=0.035$; Supplementary Figure S4), posttransplantation outcome was sensibly affected by post-induction MRD level (Figures 1d–f). Notably, SCT results were superimposable in MR and MR_1 groups (not shown), with a cumulative survival and DFS rate of 46% and 50% ($n=24$) compared with 16% and 26% in MR_2 patients ($n=19$) ($P=0.02$ and $P=0.03$), respectively. RI was 43% compared with 69% ($P=0.16$). The best overall results were observed after allo-SCT in MR/MR_1 patients, with cumulative survival and DFS rates of 60% ($n=15$) compared with 27 and 18% in MR_2 subset ($n=11$) ($P=0.08$ and 0.05), and a RI of 23% compared with 64% ($P=0.09$) (Figures 1g–i).

This very long-term update of a prospective trial included 136 MRD-evaluable patients with Ph− ALL, extending our prior observation on 112 patients with both Ph− and Ph+ disease.[2] The dominant prognostic role of MRD was confirmed even after

Table 1. Patient characteristics and MRD study results, by original risk model and quantitative MRD range

	Patients (n = *304*)		*MRD risk model*[a] (n = *136*)		*Quantitative MRD range*[b] (n = *136*)			
	Diagnosis (n = *304*)	*CR* (n = *258*)	*MRD−* (n = *76*)	*MRD+* (n = *60*)	*CMR* (n = *64*)	*MR* (n = *21*)	*MR_1* (n = *17*)	*MR_2* (n = *34*)
Age, years median (range)	35 (15.6–67.8)	33 (15.6–65.9)	30 (15.6–63.6)	37 (16.9–64.8)	30 (15.6–63.6)	32.3 (16–58.2)	37.2 (20.6–63.5)	38.6 (17.3–64.8)
Gender, M/F no. (%)	173/131 (57/43)	150/108 (58/42)	36/40 (47/53)	37/23 (62/38)	31/33 (48/52)	13/8 (62/38)	12/5 (71/29)	17/17 (50/50)
Risk group,[c] no. *(%)*								
SR B-lineage	103 (34)	89 (34.5)	36 (47)	25 (42)	30 (47)	8 (38)	7 (41)	16 (47)
HR B-lineage	108 (35.5)	89 (34.5)	22 (29)	20 (33)	19 (30)	7 (33.5)	5 (29)	11 (32)
SR T-lineage	35 (11.5)	33 (13)	9 (12)	5 (8)	7 (11)	2 (9.5)	3 (17.5)	2 (6)
HR T-lineage	58 (19)	47 (18)	9 (12)	10 (17)	8 (12)	4 (19)	2 (11.5)	5 (15)

MRD analysis,[d] no. *(%)*	*TP1*	*TP2*	*TP3*	*TP1*	*TP2*	*TP3*	*TP1*	*TP2*	*TP3*	*TP1*	*TP2*	*TP3*	*TP1*	*TP2*	*TP3*	*TP1*	*TP2*	*TP3*
CMR	60	69	75	12	11	2	58	62	63	8	9	9	3	7	4	3	2	1
MR	7	5	—	13	12	20	—	—	—	12	11	12	3	3	4	5	3	4
MR_1	2	—	—	10	15	11	—	—	—	—	—	—	11	6	9	1	9	2
MR_2	1	—	—	22	17	23	—	—	—	—	—	—	—	—	—	23	17	23
$M_{u/k}$	6	2	1	3	5	4	6	2	1	1	1	—	—	1	—	2	3	4

Abbreviations: CMR, complete molecular remission; EGIL, European Group for the Immunological Characterization of Acute Leukemias; HR, high risk; MR,, molecular remission; MR_1, molecular resistance level 1; MR_2, molecular resistance level 2; MRD, m inimal residual disease; $MRD_{u/k}$, MRD unknown; SR, standard risk; TP, time point. [a]According to original study design: MRD-negative (−) if $<10^{-4}$ at TP2 (week 16) and negative at TP3 (week 22); MRD-positive (+) with any other TP2/TP3 combination (TP1 not considered). [b]CMR, MRD negative; MR, MRD $<10^{-4}$; MR_1, MRD 10^{-4} to $<10^{-3}$; MR_2, MRD $\geqslant 10^{-3}$. [c]SR (standard risk) = leukocyte count $<30\times10^9$/l, non-pro-B/EGIL BI immunophenotype, non-adverse cytogenetics, CR after cycle 1 (B-lineage); leukocyte count $<100\times10^9$/l, cortical/EGIL T-III immunophenotype, non-adverse cytogenetics, CR after cycle 1 (T-lineage); HR (high risk) = any non-SR characteristic; adverse cytogenetics = t(4;11)/MLL rearrangement, +8, −7, del 6q, t(8;14), low hypodiploidy/near triploidy (30–39 chromosomes/60–78 chromosomes), complex karyotype ($\geqslant$3 unrelated clonal abnormalities). [d]TP, time-point.

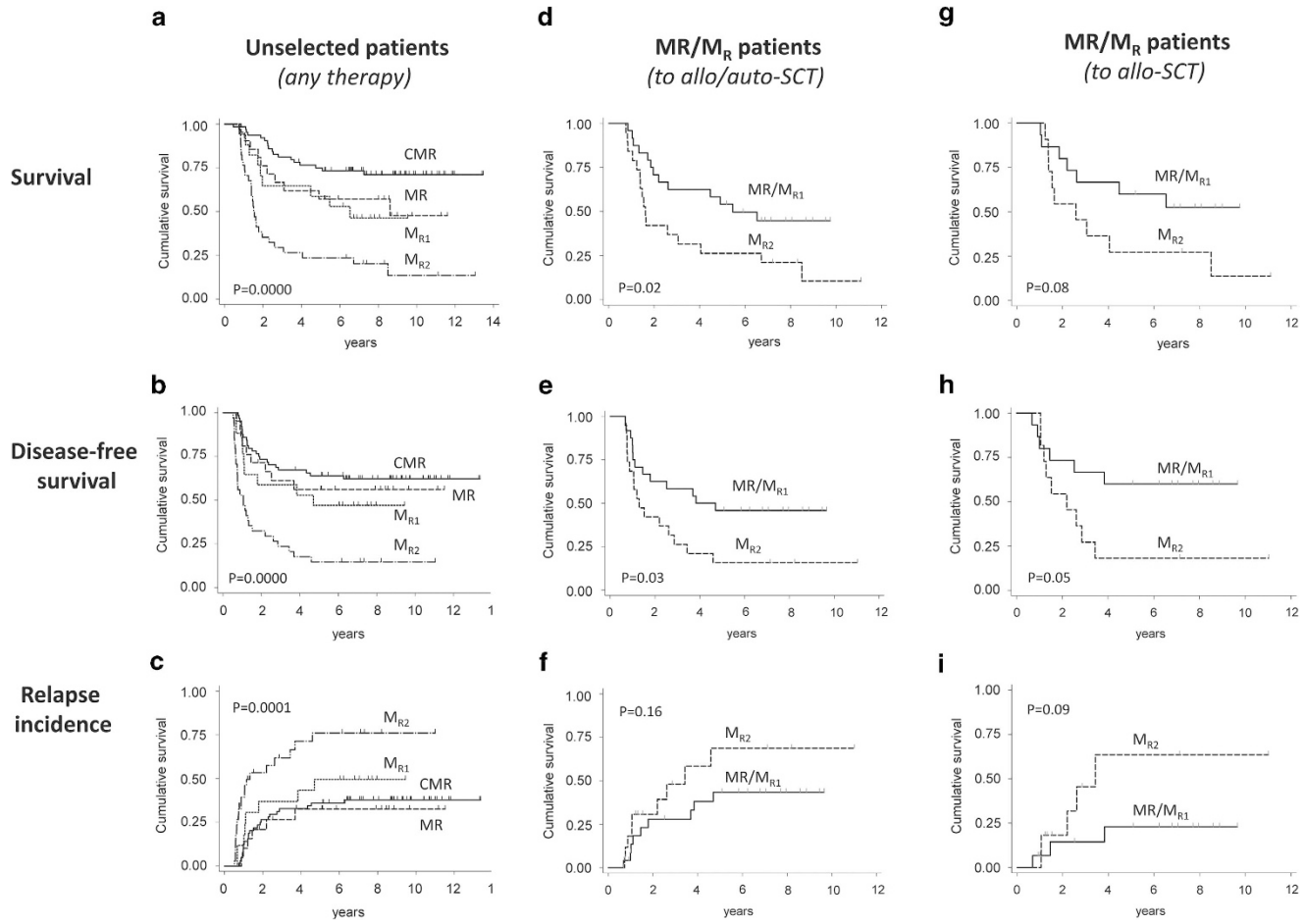

Figure 1. Outcomes by quantitative MRD ranges. Shown are long-term survival, DFS and RI rates according to MRD quantitative ranges and SCT therapy (6-year probability is given for each group). (**a**–**c**) All patients with MRD study ($n=136$): CMR ($n=64$) 0.73, 0.63, 0.36; MR ($n=21$) 0.57, 0.52, 0.33; MR_1 ($n=17$) 0.53, 0.47, 0.50; MR_2 ($n=34$), 0.24, 0.15, 0.76. (**d**–**f**), MRD+ patients receiving allo/auto-SCT ($n=43$): MR/MR_1 ($n=24$) 0.50, 0.46, 0.43; MR_2 ($n=19$) 0.26, 0.16, 0.69. (**g**–**i**) MRD+ patients receiving allo-SCT ($n=26$): MR/MR_1 ($n=15$) 0.60, 0.60, 0.23; MR_2 ($n=11$), 0.27, 0.18, 0.64.

prolonged follow-up, and the extent to which MRD+ patients were rescued by an allo-SCT correlated with post-induction quantitative MRD ranges, the allograft being performed after a median of 2.2 months from the last consolidation course. The study conclusions are that in terms of RI the outcome of patients with CMR or MR was very similar, allowing a probability of cure around 70% in patients treated with chemotherapy only because MR at TP1 and TP2, and CMR at TP3. Moreover, the patients with MRD 10^{-3} and greater (MR_2) did very badly even after an allo-SCT, although this was intentionally prescribed to overcome the high risk of relapse associated with MRD positivity. Therefore, only those patients who displayed MRD $<10^{-3}$ and were selected for transplantation because MR_1 at TP2 and/or MR/MR_1 at TP3 had a realistic chance of cure following allo-SCT, with a DFS of 60% and a RI of about 20%. These findings may be relevant to the correct positioning of SCT in MRD+ patients, including those with low-positive MRD outside the quantitative 10^{-4} cut-off.[3] This information is certainly different from that conveyed by a direct pre-transplantation MRD assay, by which we can directly compare the SCT effects with baseline.[5–7] Rather, it represents a general risk index of transplantation failure, obtainable well ahead of SCT by studying post-induction MRD, and therefore most useful for an effective SCT planning, net of several confounding factors such as the time elapsed from CR to transplantation, the intervening treatments and MRD fluctuations due to the transient efficacy of different chemotherapy courses, individual variations of dose intensity or issues of marrow sampling and MRD processing. In other reports, a post-induction MRD of 10^{-4} and greater at weeks 6, 16 and 18 was associated with a posttransplantation DFS rate of about 52% at 4 years,[8] 44% at 5 years[3] and 35% at 4 years.[4] However, these results were not further dissected by different quantitative MRD ranges.

The warning raised by our analysis is that patients with post-consolidation MRD levels of 10^{-3} and greater can have a worse posttransplantation outcome despite a justified commitment toward the procedure in view of its greater anti-leukemic power.[9] Although the general experience already indicates a higher relapse risk in MRD+ patients,[7] defining more clearly MRD thresholds associated with higher risk of failure can help design better treatment strategies. For instance, in cases with a MR_2 profile, further intensification of chemotherapy is not expected to be effective, given the saturation of the MRD response already observed at week 16 in a large German trial adopting a very intensive schedule.[3] Alternative treatments for MR_2 patients are nelarabine in T-ALL[10] and chimeric antigen receptor-modified T cells (CD19.CAR T)[11] or monoclonal antibodies in B-precursor ALL. The latter are the calecheamicin-conjugated anti-CD22 inotuzumab ozogamicin[12] and the bispecific anti-CD3/CD19 construct blinatumomab. With blinatumomab, 14 of $16MR_2$ patients (87.5%) converted to a CMR status, which in some cases lasted for >2 years without SCT.[13] A pre-emptive posttransplantation strategy with donor lymphocyte infusions or

cyclosporine A tapering should also be considered.[14,15] Adult ALL patients with high post-induction MRD (MR_2) may represent a very high-risk subset deserving close MRD monitoring and new experimental treatments aimed at reducing MRD both prior and subsequent to SCT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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