Anthracycline dose intensification improves molecular response and outcome of patients treated for core binding factor acute myeloid leukemia

Two prospective trials have demonstrated that daunorubicin used at a daily dose of 90 mg/m² over three days improved overall survival as compared to 45 mg/m² in patients with newly diagnosed acute myeloid leukemia (AML).[1,2] Benefit of intensification seems limited to the patients without adverse cytogenetics and it is still unclear whether daunorubicin 90 (DNR90) is superior to 60 mg/m² (DNR60).[3,4] Moreover, only limited data are available on the most chemo-sensitive group of patients, namely core binding factor AML (CBF-AML). In recent years, several studies have pointed out the importance of minimal residual disease (MRD) by RQ-PCR in this subgroup.[5,6] Indeed, higher MRD level after one course of consolidation chemotherapy has been proposed as the strongest factor for the prediction of relapse.[5] Here, we have retrospectively compared the effects of DNR90 *versus* DNR60 on the post-induction MRD status and outcome of 86 patients with previously untreated CBF-AML. All patients gave written consent for use of clinical and biological data. Patients were adults (age 16-65 years) diagnosed with untreated CBF-AML. All were treated by induction chemotherapy associating cytarabine 200 mg/m²/d Days 1-7 CIV with DNR 60 mg/m²/d (3 days) for patients between 2005 and 2010 or DNR 90 mg/m²/d (3 days) between 2010 and 2013. Consolidation therapy was used in 2-3 cycles of high-dose cytarabine (HDAC). Patients with less than 3 log reduction of CBF transcript after first consolidation and a fully matched donor were eligible for allogeneic transplantation. Disease evaluation was performed according to IWG 2003 criteria.[7] CBF transcript level was assessed on blood or marrow before induction (baseline), after induction (MRD1), cycle 1 (MRD2), and cycle 2 (MRD3) of consolidation. Additional data on material and methods are available in the *Online Supplementary Appendix*.

A total of 86 patients received induction chemotherapy including 57 treated with DNR60 and 29 treated with DNR90. No significant differences in pre-treatment characteristics were observed between the 2 groups (Table 1). All patients achieved a complete response (CR) and received a minimum of 2 courses of high-dose cytarabine. Fifteen patients received an allogeneic transplantation in first CR: 11 in the DNR60 group (18.6%), 4 in the DNR90 group (14%). There was a trend for a superior 2-year overall survival (OS) in the DNR90 group (DNR60 82% *vs.* DNR90 92%; *P*=0.07) (Figure 1A). This was associated with a better relapse free survival (RFS) in the DNR90 group (55% *vs.* 91%; *P*=0.003) (Figure 1B). Dose intensification seems to have a greater impact in patients with inv(16)/t(16;16) (n=57) with 2-year probability of RFS of 95% *vs.* 43%, respectively (*P*<0.001) (Figure 1C) as compared to 78% *vs.* 75% for t(8;21) patients (n=29), respectively (*P*=NS). In a multivariate model adjusted for pre-treatment variables, DNR90 remained superior to DNR60 for RFS (HR: 0.2; 95%CI: 0.03-0.51; *P*=0.006) but not for OS (HR: 0.18; 95%CI: 0.03-1.4; *P*=0.12) (*Online Supplementary Table S1*). Regarding MRD, median CBF transcript level log reduction was significantly improved in the DNR90 group after induction and consolidations (Figure 1D). Conversely, a higher percentage of patients achieved a 3 log reduction of CBF transcript (MMR) in the DNR90 group at the different time points: 37% *vs.* 66% (*P*=0.02); 76% *vs.* 93% (*P*=0.07); 84% *vs.* 100% (*P*=0.03), respectively (Figure 1E). In a multivariate model, the effect of DNR dose on the chances of achieving MMR1 remained statistically significant after adjusting for other clinical variables with an odds ratio of 3.3 (95%CI: 1.2-9.1; *P*=0.01).

Table 1. Patients' characteristics.

	Whole population	DNR60	DNR90	*P*
N.	86	57	29	
Median age (years)	41 (16-65)	42 (16-65)	41 (20-62)	0.89
Sex (M/F)	46/40 (1.1)	31/26 (1.2)	15/14 (1.1)	0.89
Therapy-related AML	15 (17%)	12 (21%)	3 (10%)	0.37
Median WBC (G/L)	15 (1-374)	17 (2-350)	10 (1-374)	0.19
Median platelet count(G/L)	44 (4-212)	65 (6-118)	44 (4-212)	0.33
Median BM blast count	63% (20-95)	64% (21-95)	59% (20-92)	0.47
Cytogenetic findings				
t(8;21) and variants	27 (31%)	20 (35%)	7 (24%)	0.34
Inv(16), t(16;16) and variants	59 (69%)	37 (65%)	22 (76%)	
Median % for RUNX1-RUNX1T1 at diagnosis	211% (69-613)	217% (70-400)	121% (69-613)	0.33
Median % for CBFB-MYH11 at diagnosis	107% (28-540)	101% (28-540)	124% (69-374)	0.16
Induction death	0	0	0	
Allogeneic transplantation in CR 1	15 (16%)	11 (18.6%)	4 (14%)	0.27
Median follow up (months)	34 m	37 m (4-93)	23 m (6-40)	

M/F: male/female; AML: acute myeloid leukemia; WBC: white blood cell count; BM: bone marrow; DNR60 DNR 90: treatment group defined by the use of daunomycin 60 mg/m²/d 3 days or daunomycin 90 mg/m²/d 3 days respectively.

This study showed that daunorubicin dose intensification was associated with an earlier and deeper molecular response, and to a reduced risk of relapse as compared to standard 60 mg/m² dose. In the initial reports on DNR90 in AML patients, respectively 89 younger patients[2] and 33 older patients[1] with CBF-AML were analyzed. There was a trend for better outcome in the groups treated with DNR90 but this was not statistically significant. In line with these observations, our study failed to demonstrate an improvement in overall survival. This may be explained by a lower dose increase of DNR in our study as compared to previous publications (DNR45 *vs.* DNR90), by the relatively small number of patients, and, more importantly, by the high proportion of patients with CBF-AML who can be salvaged by current second-line therapies.[5,6,8] Our results thus confirm that chemo-sensitivity of CBF-AML is not restricted to high-dose cytarabine[9] as already suggested by the good results of gemtuzumab in this subgroup.[10-12] Interestingly, the benefit of intensification seemed limited to patients harboring inv(16)/t(16;16). In patients with t(8;21), the observed relapse free survival was better in both groups with no significant difference between them but needs to be confirmed in a larger number of patients. Secondly, MRD assessment can be used as a valid end point for the evaluation of new induction strategies in CBF-AML. Indeed, patients in the DNR90 group showed a faster and deeper MRD reduction and achieved a higher proportion of complete molecular responses that translated into a reduced relapse incidence. Previous studies showed that

the level of MRD reduction measured early after CR through standardized techniques identifies a low relapse risk group among CBF-AML patients.[5,6] These patients represent approximately 50-70% of the total population and have a relapse incidence of under 20%. Thus, MRD assessment could be used as a valid end point for the evaluation of new induction strategies in CBF-AML. Interestingly, there was similar kinetics of MRD reduction in our study compared to the two studies mentioned above,[5,6] indicating that the administration of an intensified induction course had an effect on early MRD time points (after induction or consolidation 1). The repetition of high-dose cytarabine consolidations allowed patients treated with standard induction to finally achieve similar levels of molecular response. Consequently, it seems that only early MRD assessments were associated with outcome, unlike later time points.[6] This underlines the importance of early MRD assessment in the management of CBF-AML, and may also suggest that DNR90 could be useful for patients who may not be eligible for high-dose cytarabine consolidation, such as elderly patients[13] in whom DNR90 induction is feasible, and thus provides an opportunity to optimize molecular

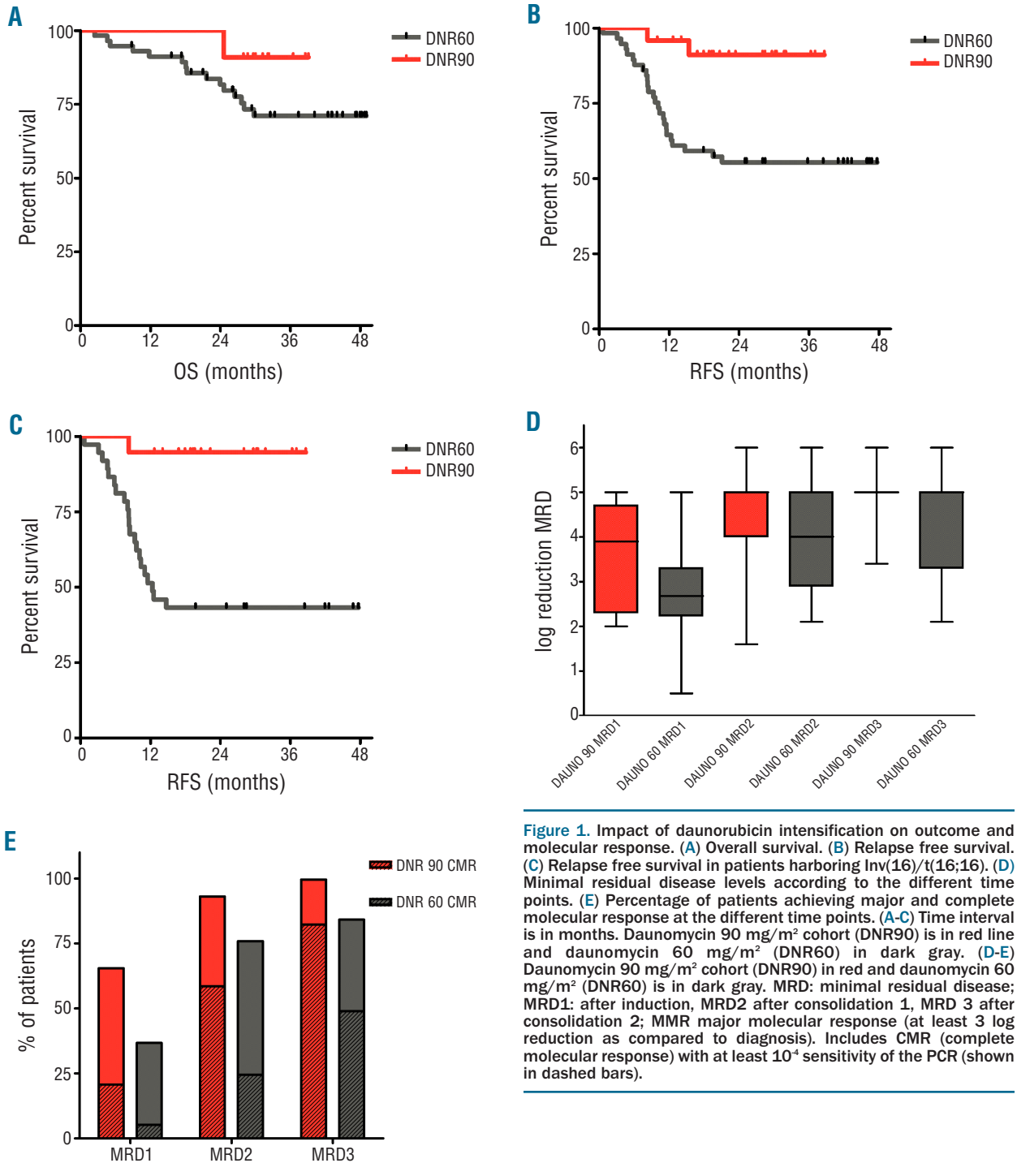

Figure 1. Impact of daunorubicin intensification on outcome and molecular response. (A) Overall survival. (B) Relapse free survival. (C) Relapse free survival in patients harboring Inv(16)/t(16;16). (D) Minimal residual disease levels according to the different time points. (E) Percentage of patients achieving major and complete molecular response at the different time points. (A-C) Time interval is in months. Daunomycin 90 mg/m^2 cohort (DNR90) is in red line and daunomycin 60 mg/m^2 (DNR60) in dark gray. (D-E) Daunomycin 90 mg/m^2 cohort (DNR90) in red and daunomycin 60 mg/m^2 (DNR60) is in dark gray. MRD: minimal residual disease; MRD1: after induction, MRD2 after consolidation 1, MRD 3 after consolidation 2; MMR major molecular response (at least 3 log reduction as compared to diagnosis). Includes CMR (complete molecular response) with at least 10^{-4} sensitivity of the PCR (shown in dashed bars).

response and, potentially, outcome. Finally, with the current intensive chemotherapy regimens, classical morphological CR rate in CBF-AML are above 90%[14] precluding the use of this criterion as an end point for the early assessment of new induction strategies in this setting. Our results, together with data from other groups, showed that molecular response might be used as a surrogate marker for relapse incidence and could be used as an end point for the investigation of new induction regimen.

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