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Delving the depths of MRD negativity in acute myeloid leukemia

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Although remission rates are high after frontline chemotherapy in acute myeloid leukemia (AML), many patients in remission will have residual leukemic cells that may initiate relapse if not cleared sufficiently by further therapy. The advent of measurable residual disease assays for AML has resulted in more sensitive estimates of residual leukemia, allowing patients to be subdivided into complete morphological remission with negative MRD (CR_{MRD}^-) or with positive MRD ($CR_{MRD}+$)[1]. These response categories have implications for therapeutic decisions as AML patients with an MRD negative remission have substantially better outcomes- independently of other factors such as their genetic risk - with an average hazard ratio of 0.36 and 5-year overall survival of 68% from a large meta-analysis of 11,151 patients[2]. Indeed, several AML trial groups have progressed from validation of MRD as a key prognostic marker to clinical trials that use MRD results to direct therapy. Not surprisingly such trials have predominantly targeted younger adults with intermediate genetic risk AML in first remission for MRD guided strategies. This is due to the perceived need for better risk stratification in this group to inform decisions on allogeneic transplant. Whether intermediate risk younger adults with a CR_{MRD}^- test after 1 or more courses can be spared the toxicity of allogeneic transplant without a detrimental effect on their survival is now a central question for the management of AML. Evidence to support this approach has recently emerged from the GIMEMA-AML-1310[3] and HOVON-SAKK-132[4] trials that allocated intermediate risk younger adults with a CR_{MRD}^- test, as assessed by flow cytometry after 2 courses, to autologous rather than allogeneic transplant. Both trials reported encouraging 2-year survival rates of over 75% for these patients when they received their autologous transplant.

Now an extended analysis of the GIMEMA-AML-1310 trial by Dr Buccisano and colleagues in this issue[5] sheds light on whether current ELN criteria of a flow cytometric MRD-negative test (<0.1% of leukocytes) can be refined to identify patients with a deeper remission and, crucially, whether these 'deeper' responders have significantly better outcomes. In AML, there is a high level evidence base and agreement that flow cytometric MRD of 0.1% or above correlates with high relapse rates and inferior survival at

multiple treatment time-points[1]; this includes intermediate risk younger adults with wild type *NPM1* mutations when MRD is measured after the first 2 chemotherapy courses (cumulative incidence of relapse at 3 years of 89%)[6]. Flow cytometric MRD below 0.1% may represent technically detectable as well as undetectable residual leukemia commensurate with assay sensitivity and accordingly has less well defined prognostic relevance (Figure*)*. By the technical /statistical parameters of rare event analysis (set by acceptable coefficients of variation for reliability of the measurement), flow cytometric MRD is undetectable with less than 20-30 positive cells and unquantifiable with less than 50. These standardised criteria for the limit of detection (LOD) and quantitation (LOQ) are applied to report high sensitivity flow cytometric MRD in myeloma[7], chronic lymphocytic leukemia (CLL)[8] and acute lymphoblastic leukemia (ALL)[9] following extensive clinical validation. The GIMEMA investigators sought to establish their prognostic value in AML. Firstly they observed that only two thirds of patients with MRD negative tests (categorised in the AML-1310 trial as below 0.035% after 2 or 3 courses) had deeper remissions by the LOQ (ie <0.01% MRD cells of 0.5 million leukocytes). Then importantly, they show this LOQ further discriminated survival in the overall MRD negative group (as categorised by the trial). Those identified as achieving a deeper remission by the LOQ criteria had a 2-year survival of 86.7% compared to 72.5% for the remaining CR_{MRD}^{-} adults ($p<0.01$). Restricting the analysis to the intermediate genetic risk group produced similar results. These reproducible flow cytometric criteria may therefore improve prognostic information in AML by identifying at least some patients with a deeper remission. This new information paves the way for standardised improved reporting of flow cytometric AML MRD and, in parallel, prompts questions on how the results might be used to further guide targeted de-escalation or intensification of therapy.

While some AML-1310 patients were re-classified as MRD-positive from the LOQ thresholds, they had a non-inferior outcome to those with MRD-positivity over 0.035% (2-year survival of 72.5% and 67% respectively), despite not having been identified for MRD-directed allogeneic transplantation. This supports the current consensus that intensification cannot be recommended simply based on persisting low level MRD after frontline treatment, particularly when levels are stable in serial measurements. Of course the prognostic value of low level MRD may vary according to treatment schedules and genetic risk but, importantly, accurate estimation of this will depend on the robust exclusion of technical false positives (arising from background). This is being addressed in ongoing initiatives by the ELN-DAVID group and others.

Conversely, given the excellent survival of the deep responders by LOQ criteria, could separating out these patients be a first step to sparing them unnecessary intensification or maintenance? It is of interest that about 40% of the AML-1310 cohort with *Flt3-ITD* mutations or poor risk cytogenetics were in the 'deeper' responder category. With regards the former, if the AML is also *NPM1* mutated, accumulated evidence supports the strategy of serial PCR MRD monitoring for deep responders[1]. This will enable the

toxicity of an allogeneic or even an autologous transplant to be avoided for some patients. A similar watch-and-wait approach could be extended to other intermediate risk patients using serial flow cytometric MRD monitoring. For younger adults with allo-mandatory AML, the balance of benefit for myeloablative versus reduced intensity conditioning remains controversial[10]. An early deep MRD response sustained at the pre-transplant MRD assessment could more precisely identify those patients for whom reduced intensity conditioning may suffice to prevent relapse[11].

Based on this study, flow cytometric MRD response measurements that incorporate the absolute flow cytometric LOQ thresholds - already in use for myeloma, CLL and ALL - have promise as an useful adjunct to extend the current ELN recommended flow cytometric definition of CR_{MRD}^{-} for AML. Consideration should be given to the collection of this data in ongoing trials to improve interpretation of treatment efficacy.

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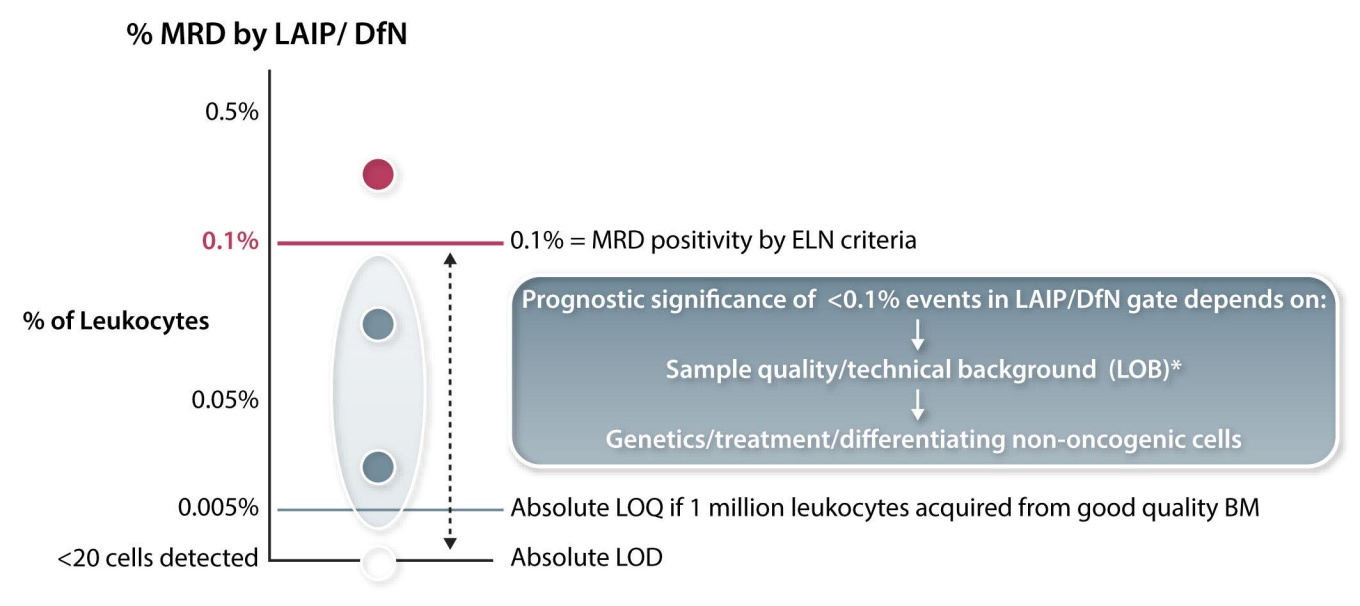
Figure. Interpretation of Flow Cytometric MRD

*More than 50 cells (absolute LOQ) in the AML leukemic aberrant immunophenotype gate may constitute background noise. The level of background noise (LOB) from non-leukemic blasts will depend on the exclusion of normal regenerating blasts by the LAIP gate and can be estimated by testing a range of control BMs. For example if LAIP gate has a background noise of up to 0.02% and 0.5 million leukocytes are acquired, there may be up to 100 non- AML cells.

Abbreviations: MRD, measurable residual disease; LAIP, leukemic aberrant immunophenotype; DFN, different from normal aberrant immunophenotype; LOD, limit of detection; LOQ, limit of quantitation; LOB, limit of blank; BM, bone marrow


% MRD by LAIP/ DfN
0.5%
0.1%
0.1% = MRD positivity by ELN criteria
% of Leukocytes
0.05%
Prognostic significance of <0.1% events in LAIP/DfN gate depends on:
Sample quality/technical background (LOB)*
Genetics/treatment/differentiating non-oncogenic cells
0.005%
Absolute LOQ if 1 million leukocytes acquired from good quality BM
<20 cells detected
Absolute LOD