Delving the depths of measurable residual disease negativity in acute myeloid leukemia

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https://doi.org/10.3324/haematol.2022.280747

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 (MRD) assays for AML has resulted in more sensitive estimates of residual leukemia, allowing patients to be subdivided into those with 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% according to 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 transplantation. Whether intermediate-risk younger adults with a CR_{MRD-} test after one or more courses can be spared the toxicity of an 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 two courses, to autologous rather than allogeneic transplantation. Both trials documented 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 Buccisano and colleagues, published in this issue of *Haematologica*,[5] sheds light on whether current European LeukemiaNet (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

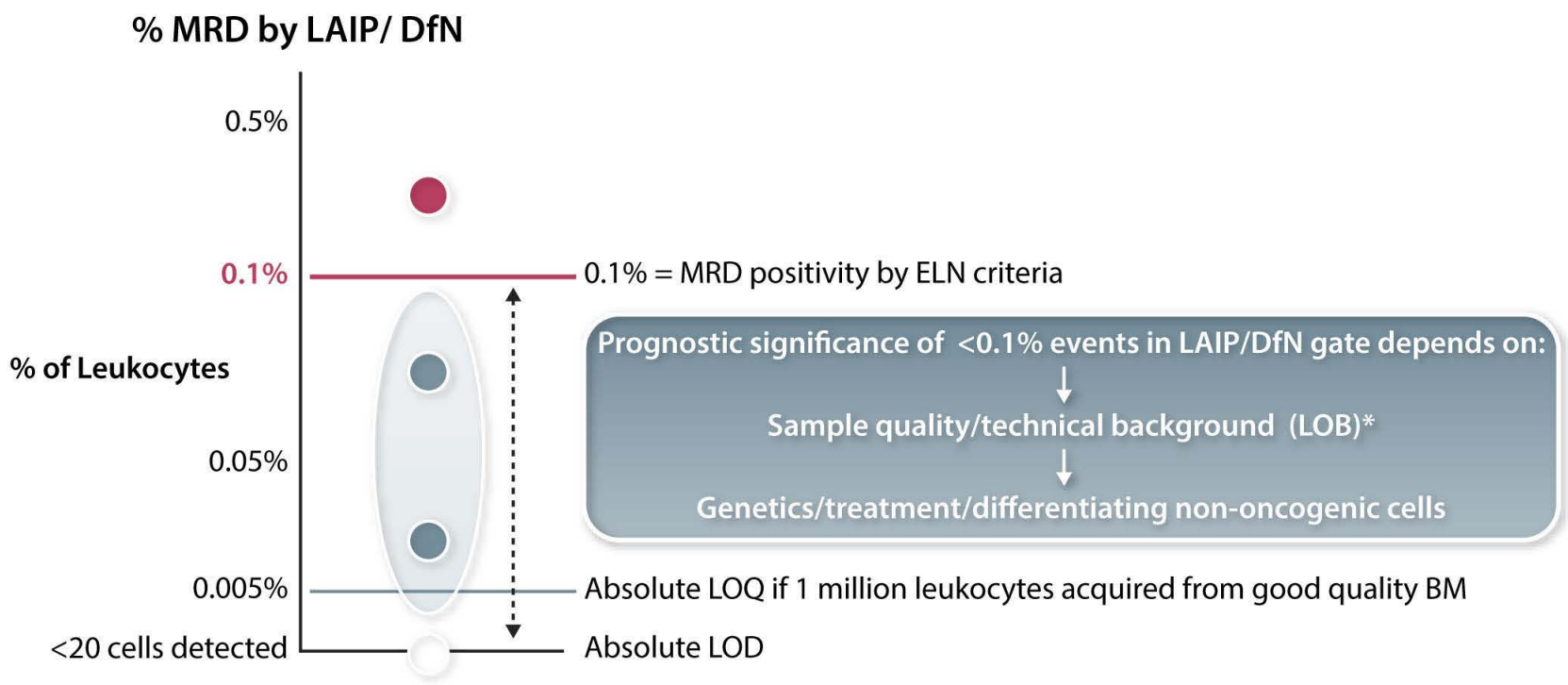

Figure 1. Interpretation of flow cytometric measurable residual disease. *More than 50 cells (absolute limit of quantification in the acute myeloid leukemic aberrant immunophenotype (LAIP) gate may constitute background noise. The level of background noise from non-leukemic blasts will depend on the exclusion of normal regenerating blasts by the LAIP gate and can be estimated by testing a range of control bone marrow samples For example, if the LAIP gate has a background noise of up to 0.02% and $0.5x10^6$ leukocytes are acquired, there may be up to 100 non-acute myeloid leukemia cells. MRD: measurable residual disease; LAIP: leukemic aberrant immunophenotype; DfN; different from normal aberrant immunophenotype; ELN: European LeukemiaaNet; LOB: limit of blank; BM: bone marrow; LOD: limit of detection; LOQ: limit of quantitation.

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 two 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 1). 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 standardized criteria for the limit of detection (LOD) and quantitation (LOQ) are applied to report high sensitivity flow cytometric MRD in multiple myeloma,[7] chronic lymphocytic leukemia[8] and acute lymphoblastic leukemia[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 (categorized in the AML-1310 trial as below 0.035% after 2 or 3 courses) had deeper remissions by the LOQ (i.e., <0.01% MRD cells of $0.5x10^6$ leukocytes). Then, importantly, they showed that this LOQ further discriminated survival in the overall MRD-negative group (as categorized 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 standardized, 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 patients in AML-1310 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 regard to the former, if the AML is also *NPM1*-mutated, accumulated evidence supports the strategy of serial polymerase chain reaction 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 AML in whom allogeneic transplantation is mandatory, 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 multiple myeloma, chronic lymphocytic leukemia and acute lymphoblastic leukemia - have promise as a useful adjunct to extend the current ELN recommended flow cytometric definition of CR_{MRD-} for AML. Consideration should be given to the collection of these data in ongoing trials to improve interpretation of treatment efficacy.

Disclosures

Speakers bureau for Jazz and Novartis; consultancy or advisory role for Novartis and Neogenomics; research funding from Jazz and BMS.

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