Molecular measurable residual disease: staring at red herrings

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Red herrings, misleading or distracting clues, have been utilized in some of the most famous works of literature and film to maximum dramatic effect. The term is thought to have originated from the practice of using the pungent odor of cured fish to distract young hunting hounds in training; the more they learned to ignore the stench of the herring (which turned red in the process of being smoked), the better they were able to hone their focus on the scent of their prey.

The presence of measurable residual disease (MRD) in patients achieving morphologic remission post-treatment is one of the most powerful predictors of acute myeloid leukemia (AML) relapse(1); it is not at all a red herring. Although conventionally performed via multi-parameter flow cytometry, MRD can also be assessed through a variety of high-sensitivity molecular tools to query allelic frequency of AML-associated genes(2). With respect to this so-called “molecular MRD,” given its potential as a more sensitive measure of residual disease, we must grapple with the question of when an assay’s depth is too deep, and when detectable gene mutations might not herald looming relapse but instead represent red herrings. Previous literature has provided strong evidence that clonal hematopoiesis mutations DNMT3A, TET2, and ASXL1 (“DTA” mutations, or, we might propose, “RH” mutations) may persist after therapy and are not associated with increased relapse risk(3). In the current issue of *Haematologica*, Murphy and colleagues describe an unbiased mathematical approach to evaluating the contribution of individual genes toward the predictive value of MRD.

In their letter entitled, “Exclusion of persistent mutations in splicing factor genes and IDH2 improves the prognostic power of next-generation sequencing (NGS)-based MRD assessment in acute myeloid leukemia,” this group evaluated persistence of mutations in 22 AML-associated genes in remission samples from 101 patients who received standard cytotoxic chemotherapy for newly diagnosed AML(4). In most cases two separate remission samples were evaluated with error-corrected NGS. The authors used a conservative mutant allelic frequency (MAF) cutoff of 1% to categorize patients as MRD positive versus negative. They then systematically excluded individual genes from MRD analysis within the cohort, yielding 2500 permutations of MRD for which the hazard ratio (HR) for overall survival (OS) was calculated and compared. Their conclusions were that, in addition to DTA mutations, exclusion of splicing factor mutations (SRSF2, U2AF1, and SF3B1) and IDH2 enhanced the predictive value of MRD for OS as well as relapse-free survival (RFS) and cumulative incidence of relapse (CIR). They went on to validate these findings in two historic cohorts of patients for whom NGS MRD data were
available, showing that removal of “DTASI2” mutations from MRD evaluation enhanced the prognostic value of the assay.

The approach taken by the investigators is novel and does attempt to mitigate bias inherent in much of the existing molecular MRD literature. The clinical outcome is the true measure of a gene’s value for MRD, if molecular MRD is being utilized as a purely clinical assay without reference to its research value in imputing clonal dynamics of disease. It is interesting that the authors chose OS as their endpoint, rather than RFS, since residual disease is by definition a predictor of relapse, whereas the contributors to OS are multifactorial in the adult population. The 1% MAF cutoff also raises questions about the validity of these findings at lower thresholds such as 0.1% or 0.02%, which are more commonly used clinically as positive/negative cutoffs. It is true that the accepted thresholds for NGS MRD have yet to be established(2) and using a higher MAF burden for thresholding is more likely to capture more proximal survival events. However, it is possible that a lower MAF threshold would allow for even better discrimination between outcomes.

The removal of splicing factor mutations as a class from consideration for AML MRD is supported by prior studies demonstrating their association with pre-leukemic marrow disease, particularly myelodysplastic syndrome (MDS). Their persistence after both conventional chemotherapy and epigenetic agents has also been described and was not associated with inferior survival(5, 6). Therefore, the present findings add further credence to their exclusion from molecular MRD assessments. Similarly, DNMT3A and TET2 are again confirmed to lessen the predictive value of molecular MRD by their exclusion, although it is interesting to note that ASXL1 was not among the genes highlighted in Figure 1A or 1B as worthy of exclusion in the mathematical modeling, but was excluded nonetheless by convention(2, 3). The exclusion of IDH2 is more controversial. While the authors show optimal hazard ratios for OS with “DTASI2” genes excluded, they do not directly compare these hazard ratios to “DTAS” alone to show specifically that IDH2 exclusion enhances prognostic value of molecular MRD. Furthermore, based on the heatmap in Figure 1A, there is not only a cluster of IDH2 exclusion at the high hazard ratio end of the ranked permutations, but another cluster at the low hazard ratio end as well. This pattern is not seen with DNMT3A, TET2, or splicing factor mutations – or indeed any other gene in the panel. This may reflect different contributions of IDH2 to clonal evolution in individual patients. While mutations in IDH2 are known to be necessary but not sufficient for leukemic transformation in preclinical models(7) and have also been described as early mutations in MDS and pre-leukemic myeloproliferative disorders(6, 8), there are numerous reports of IDH2 being used to successfully monitor MRD(9). It may be that IDH2 is a founder event in some patients, and therefore analogous to clonal hematopoiesis or splicing factor mutations in its lack of prognostic value for relapse(6, 10), whereas in other instances of AML it is a later mutation, and therefore still useful for MRD monitoring. Additional studies will be necessary to reproduce the current findings in larger cohorts, paying particular attention to the co-mutations and putative clonal evolution in individual patients.

Despite these caveats, the authors are to be commended for their a priori approach to mutation evaluation for MRD relevance in AML treated with conventional induction therapies. In addition to prospective validation of these findings in a similar context, ongoing work should evaluate the
utility of individual genes for molecular MRD monitoring after low-intensity therapies such as venetoclax based regimens, as these therapies are gaining ground in particular AML populations, but very little is understood about their effect on clonal dynamics or molecular MRD. As the field gets closer to adoption of gene-based MRD for clinical decision-making, we will need stringent systems in place to filter out gene mutations whose persistence smells fishy.

References


Figure 1 According to Murphy et al., inclusion of DNMT3A, TET2, ASXL1, splicing factor genes (SRSF2, SF3B1, U2AF1), and IDH2 mutations (DTASI2) in measurable residual disease (MRD) quantitation for AML may worsen the prognostic value of MRD in this context. DTASI2 mutations may be akin to red herrings that distract or mislead clinicians in their assessments of true AML residual disease.