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 films 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 morphological remission after 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 multiparameter flow cytometry, MRD can also be assessed through a variety of high-sensitivity molecular tools to investigate the 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 isocitrate dehydrogenase 2 improves the prognostic power of molecular measurable residual disease 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 next-generation sequencing. The authors used a conservative mutant allelic frequency (MAF) cutoff of 1% to categorize patients as MRD-positive or MRD-negative. They then systematically excluded individual genes from the MRD analysis within the cohort, yielding 2,500 permutations of MRD for which the hazard ratio for overall survival 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 overall survival as well as relapse-free survival and cumulative incidence of relapse. They went on to validate these findings in two historic cohorts of patients for whom next-generation sequencing 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 yardstick 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 overall survival as their endpoint, rather than relapse-free survival, since residual disease is by definition a predictor of relapse, whereas the contributors to overall survival are multifactorial in the adult population. The 1% MAF cutoff also raises questions about the validity of the 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 next-generation sequencing 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 mar-

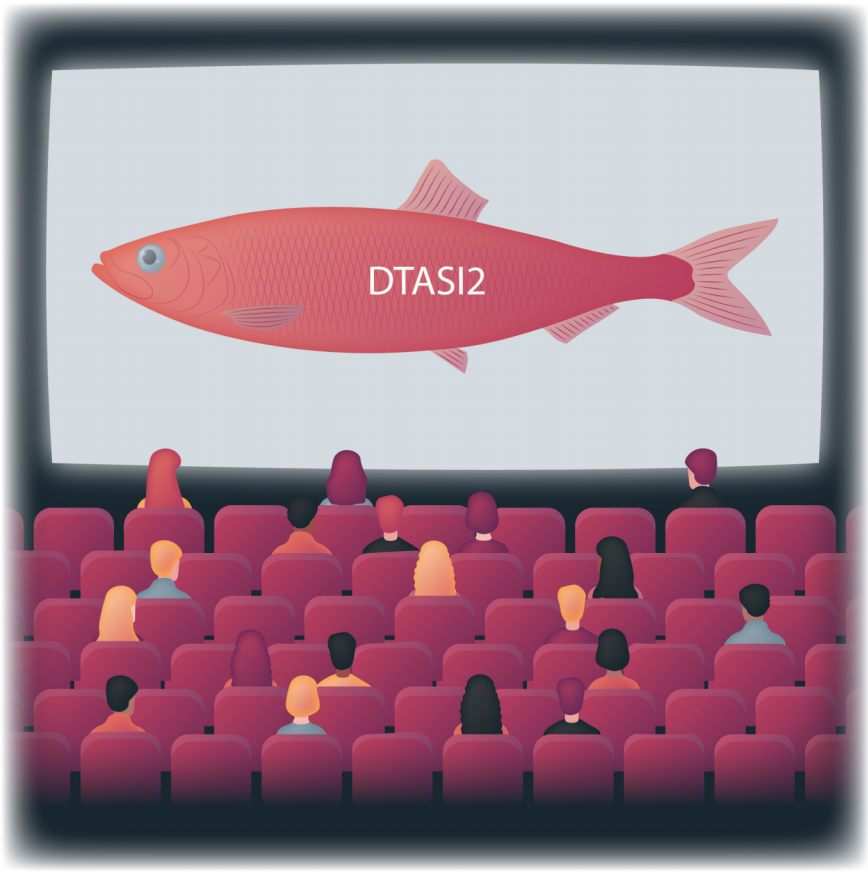

Figure 1. A fishy story. According to Murphy *et al.*[4], inclusion of *DNMT3A*, *TET2*, *ASXL1*, splicing factor genes (*SRSF2*, *SF3B1*, *U2AF1*), and *IDH2* mutations (DTASI2) in measurable residual disease quantitation for acute myeloid leukemia may worsen the prognostic value of measurable residual disease in this context. DTASI2 mutations may be akin to red herrings that distract or mislead clinicians in their assessments of true residual disease in acute myeloid leukemia.

row disease, particularly myelodysplastic syndromes. 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 of Murphy's publication 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 overall survival with "DTASI2" genes excluded, they do not directly compare these hazard ratios to "DTAS" alone to show specifically that the exclusion of *IDH2* enhances the prognostic value of molecular MRD. Furthermore, based on the heatmap in Figure 1A of the letter by Murphy *et al.*, 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 myelodysplastic syndromes 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 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 patients 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.

Disclosures

No conflicts of interests to disclose.

Contributions

ACW and DAP wrote and edited this editorial.

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