Mutant allelic burden in acute myeloid leukaemia: Why bother?

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In this issue of the journal, David Linch and colleagues present a comprehensive analysis on the NPM1 mutant allele frequency in a large cohort (n = 876) of younger acute myeloid leukaemia (AML) patients (Linch *et al.*, 2019). The significance of the *NPM1* mutant allelic burden for prediction of clinical outcome in AML had previously been addressed in smaller cohorts of patients, leading to conflicting conclusions. While Patel *et al* (2018) proposed that a high *NPM1* mutant variant allele frequency (VAF) at diagnosis predicts an unfavourable outcome of treatment, which they more recently extended by demonstrating that this correlates with minimal residual disease status in first remission (Patel *et al.*, 2019), other comparable studies showed that *NPM1* mutant VAFs correlated with leukaemia burden but not with survival (Abbas *et al.*, 2019). In line with this latter publication, the data presented by Linch *et al* (2019) also show that different levels of the *NPM1* mutational burden do not have predictive value for complete remission (CR) and overall survival rates in a multivariate analysis. This also applied to patients receiving an allograft in first CR, a subset of patients in which high *NPM1* VAF had a particularly adverse impact in the studies reported by Patel *et al* (2018, 2019).

What lessons can we learn from these apparent controversies? First of all, it is relevant to ask what the reported differences in VAF really meant. Because DNA was isolated from bone marrow or blood buffy coat cells and percentages of AML blasts in the test samples were not reported, variable admixture of non-leukaemic cells probably affected the VAFs and prohibited accurate estimations of leukaemic burdens. Hence, it cannot be reliably concluded whether the VAF cut-offs to discriminate subgroups reflected levels of (sub-)clonality, normal cell admixture or a combination of these. Conceivably, discrepancies between the studies may be partly explained by differences in sample purities. Irrespective of the underlying explanation, the recommendation of Linch *et al* (2019), that the binary presence or absence of an *NPM1* mutation rather than variations in VAFs is the most robust and should therefore preferably be used in therapeutic management makes sense, also because inter-laboratory variations in sample preparations will make VAF cut-offs ambiguous.

A more general question of interest is how clonal burdens will impact on the efficacy of new therapeutic agents targeting substrates with specific mutations. Currently, such trials are underway with inhibitors of mutant isocitrate dehydrogenase 1 and 2 (*IDH1* and *IDH2*) and in AML cases with mutations or internal tandem duplications (ITDs) in *FLT3*, encoding FMS-like tyrosine kinase 3 (FLT3). Without going into details of patient selection criteria and setup of these trials, it is obvious that clonal burdens of the mutations within the AML blast population at initiation of treatment predictably have an impact on responses to these inhibitors. In cases with clonal heterozygous or homozygous mutations (VAFs ~ 50% or more), the choice for using targeting drugs is obvious. In contrast, in cases with subclonal mutations in *IDH1/2* or *FLT3*, criteria for making this choice are less clear. If VAFs reveal that mutant subclones are minor, let's say below 20%, it is unlikely that the drugs will exert a measurable therapeutic effect.

But what about patients in which major mutant subclones are present? Here, several lines of reasoning can be followed. One may predict that targeting these clones will only have a short-term clinical impact by reducing tumour load, but will not erase the founder AML clone and thus fail to contribute to durable responses. On the other hand, it can be argued that reducing these subclones might be of longer-term therapeutic benefit, for instance because these subclones could trigger bone marrow niche components to promote growth and survival of the entire AML blast population. From a more negative perspective, it is equally possible that targeting major subclones may give more niche space for *IDH1/2* or *FLT3*-ITD mutant-negative resistant founder clones and thus have an adverse effect on durable remissions.

Whether the current and future trials will provide meaningful answers to discriminate between these possibilities will depend on how patients will be stratified based on VAFs, essentially along the lines presented by Linch *et al* (2019).

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However, here it will be essential to use pure (>90%) AML blasts for the analysis to avoid normal cell admixture as a major confounder. While these trials are underway, parallel studies in patient-derived xenograft models might give additional clues as to whether targeting AML with various levels of subclonality will have clinical benefit.

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