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Persistence of DNMT3A does not influence clinical outcome in AML

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The advent of next-generation sequencing (NGS) has led to the way to the identification of a plethora of additional mutations in acute myeloid leukaemia (AML). These mutations, genetic and epigenetic alterations, have all been reported to positively or negatively associate with AML outcome. Some of the mutations identified prior to NGS have been extensively studied, such the internal tandem duplication (ITD) of the FLT3 gene, mutations of the NPM1 or CEPBA genes and have been included in the WHO classification of AML as defining specific sub-categories of patients with normal karyotypes.

The DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A) gene was identified by NGS and was shown to be mutated in approximately 20-35% of patients(Ley *et al*, 2010); this places it as one of the most frequently mutated genes in AML (Thol *et al*, 2011). The majority of mutations occur in the methyltransferase domain and involves the R882 codon

The value of determining minimal residual disease (MRD) levels has been clearly demonstrated in blood cancers notably with the regular monitoring of the BCR-ABL levels in chronic myeloid leukaemia (CML) (Hanfstein *et al*, 2012) and PML-RARa in acute promyelocytic leukaemia (APL)(Grimwade *et al*, 2015). In each of these diseases, the failure to reduce the leukaemic clone burden below the level of conventional PCR detection or an increasing level of the fusion genes has been shown to be associated with the impending onset of disease resistance or clonal evolution. These signs could be used to alter therapeutic options to improve the patient's outcome trajectory.

The relevance of the prognostic significance of persistent DNMT3A mutations has been examined in four studies prior to the one reported in this issue. Two have reported that the presence of DNMT3A mutations is associated with a poor clinical outcome (Hou *et al*, 2012;Klco *et al*, 2015); whilst the other two reported no difference in outcome (Debarri *et al*, 2015;Ploen *et al*, 2014). There are also differences in the size of the sample population, detection methodologies and the type of mutations analyzed

To clarify this situation, Bhatnagar and colleagues have reported in this issue (REF) a study on a larer cohort of AML patients who had a dominant clone with DNMT3A R882 mutation at diagnosis. All the

patients received intensive induction treatment within Cancer and Leukemia Group B (CALGB) trial protocols. Additional samples were then obtained when the patients had achieved a complete remission (CR) and both the diagnostic and CR samples were analyzed using a targeted NGS approach covering 35 genes. For the determination of positivity of DNMT3A mutations, a detection level cut-off of >3% was used. It should be noted that this study only included patients with a R882 mutation in DNMT3A and the influence of other mutations were not assessed.

At diagnosis, all the patients had at least one mutation in addition to the DNMT3A; with the level of DNMT3A mutation between 40-53%. However, the interesting aspect was observed after patients had obtained CR where two cohorts were identified: in approximately 24% of patients (cohort 1) no mutations were; whilst in cohort 2 (76%) a DNMT3A mutation persisted beyond CR with levels ranging from 3% to 45% Surprisingly, there was no significant difference between overall survival (OS) or disease-free survival (DFS) between cohort 1 and 2; irrespective of the time that the CR was taken post morphological confirmation of CR or if the sample was within 30 day of CR being confirmed. This is contrast to expectation from studies on CML and APL where the clearance or non-detection of a mutation, albeit a fusion gene rather than a point mutation, is highly significant in terms of predicting outcome. However, the persistence of DNMT3A R882 mutation post-CR appears not to have any impact on outcome.

Within cohort 2, DNMT3a mutations alone were detected in around 3/4 (~73%) of the patients in the second cohort (cohort 2a); with the remaining 12 patients (~27%) having a DNMT3A mutation and one additional mutation (cohort 2b) mainly TET2, p53 or ASXL1. Although no significant difference was seen for OS between cohorts 2a and 2b, the author report a “trend” (p=0.06) for patients with additional mutations post-CR to have poorer DFS.

To complicate the situation further, the second mutation detected in patients in cohort 2b was different from any mutation identified, in that patient, at diagnosis in 50% of these cases; indicative of clonal evolution and expansion although the whether these additional mutations contributed to relapse was not investigated.

Whilst this study has shown that persistence of mutations, at least for DNMT3A R882, has no or little impact on patient outcome it also raises questions around MRD monitoring. For example, it has demonstrated that the use of NGS at <3% detection level is not viable for MRD so perhaps less (or even more) sensitive methods, including conventional PCR, would enable discrimination between clearance and persistence in terms of outcome. Furthermore, the presence of DNMT3A mutations have been detected in healthy individuals with clonal haematopoiesis, increasing with age; so it is

possible that their persistence demonstrated here is a reflection of “normal” haematopoiesis. However, the fact that the percent of patient’s disease free or alive at 3 or 5 years was similar irrespective of DNMT3A R882 clearance or persistence highlights that AML is not only clinically and biologically heterogeneous at diagnosis but this complexity is maintained throughout disease progression.

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