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The clinical significance of *FLT3-ITD* allele burden in AML; a long-term retrospective single centre study

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INTRODUCTION

FMS-like tyrosine kinase 3 ligand (*FLT3*)-internal tandem duplication (ITD) is an important prognostic marker in patients with acute myeloid leukaemia (AML). One of the most common mutations in AML, it represents a particular clinical challenge, conferring a higher risk of treatment failure and a poor prognosis. There is increasing evidence to suggest a patient's *FLT3-ITD* allelic burden may be able to further refine this prognostic information and potentially influence treatment decisions especially with the introduction of FLT3 inhibitors.

AIM

We studied all newly diagnosed *FLT3-ITD* mutated AML cases with the aim of correlating *FLT3-ITD* allelic burden with long-term clinical outcome and to assess the utility of incorporating allelic burden ratios into our integrated diagnostic reports.

METHOD

FLT3-ITD mutated AML cases were identified over a nine-year time period (2007 to 2016) to ensure a minimum of 36 months follow up data.

FLT3-ITD, *FLT3-D835* and *NPM1* mutations were detected by multiplex PCR followed by capillary electrophoresis on peripheral blood and bone marrow samples. The size of the *FLT3-ITD* was recorded. Allelic ratios were retrospectively calculated (area *FLT3*-ITD/(area *FLT3* WT + area *FLT3*-ITD) X 100); with a high allelic ratio defined as >50% mutant to wild-type.

Patient demographics, presentation details, cytogenetic and molecular data, management and clinical outcomes were collected.

PATIENT CHARACTERISTICS

31 patients were identified as *FLT3-ITD* mutated AML, 4 of whom had PML-RARA positive acute promyelocytic leukaemia (APL). 26 patients (83.9%) were treated with intensive chemotherapy. The intensive treatment group comprised of 11 men and 15 women with an age range at diagnosis of 21-78 (median of 51). 15 patients were also *NPM1* positive, other concurrent mutations included *DEK/NUP214* (n=1). Cytogenetic results were available on 21 patients; 20 intermediate and 1 favourable risk. The majority of patients were treated out with a clinical trial (6 patients enrolled in NCRI AML17 and 1 in AML15) with the most common induction regimen being DA (n=19), followed by ATRA+Ida (n=4), FLAG-Ida (n=2) and ADE (n=1). 8 patients underwent allogenic stem cell transplant. No patients received a FLT3 inhibitor as these data were collected prior to the approval of FLT3 inhibitors in routine clinical practice.

RESULTS

The median *FLT3-ITD* allelic ratio was 47% (range 4-99%). 9 patients (34.6%) had a high *FLT3-ITD* allelic ratio (range 50.1%-99.7%; median 63.9%). There was a significant age difference between groups: the high allelic ratio group (mean: 38.6, SD: 13.0) was younger than the low allelic ratio group (mean: 60.1, SD: 11.5); p<0.01. Those with a high ratio were more likely to present with WBC >100x10^9/L (high: 33%, low: 18%). However, there was minimal difference between rates of complete response after their first cycle of induction chemotherapy (high: 89%, low: 82%) and non-significant difference in 2-year relapse free survival (high: 44%, low: 53%, p=0.95). Interestingly, patients with both *FLT3-ITD* and *NPM1* mutations a high allelic ratio did not predict an inferior 2-year relapse free survival.

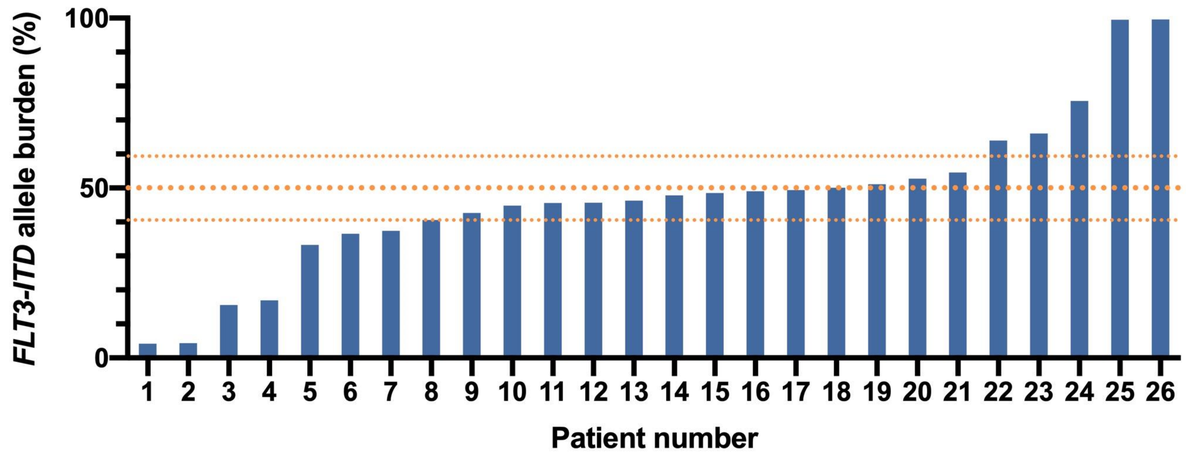

Figure 1. Distribution of *FLT3-ITD* allele burden for those who underwent treatment with intensive chemotherapy. Note samples clustered around 50%; 14 of 26 with a allele burden of 40-60%. This highlights a potential limitation of detecting difference between the two groups and in using a *FLT3-ITD*$^{high/low}$ to risk stratify patients.

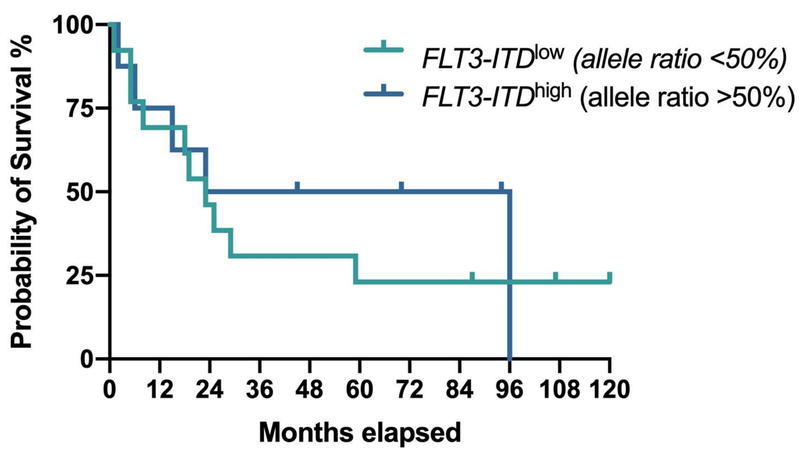

Figure 2. Kaplin-Meier survival curves comparing low and high *FLT3-ITD* allele burden for those who underwent treatment with intensive chemotherapy for AML (APL cases excluded). Survival did not differ between the two groups (Log-rank test; χ^2 (1,N=22)=0.22, p=0.64).

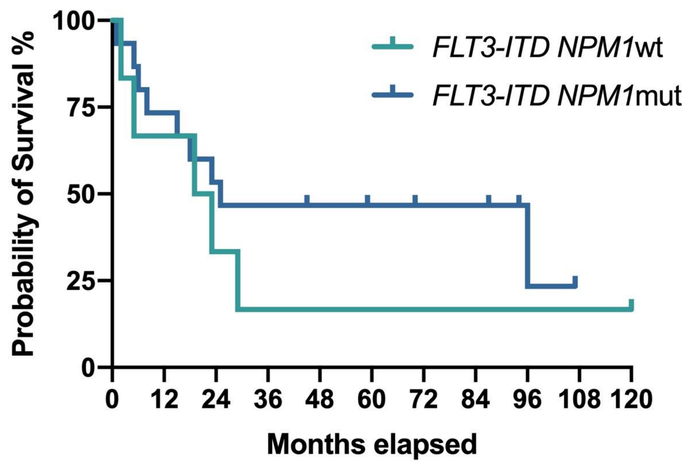

Figure 3. Kaplin-Meier survival curves comparing *FLT3-ITD* mutated AML (APL cases excluded) with NPM1 wild type and NPM1 mutated. Survival did not differ between the two groups (Log-rank test; χ^2 (1,N=22)=0.62, p=0.43).

CONCLUSIONS

Analysis of this retrospective single centre cohort was not able to demonstrate any meaningful differences between the patients with high and low *FLT3-ITD* allele burden AML. As a result, *FLT3-ITD* allele burden is not routinely reported.

Given the multitude of methods and lack of national or internationalised standardisation for *FLT3-ITD* testing this study underpins the importance of institutional validation before the integration of allele burden to routine diagnostic reporting and clinical risk assessment.

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