

# ***Prognostic models turn the Heat(IT)up on FLT3<sup>ITD</sup> mutated AML.***

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## Abstract

The presence of internal tandem duplications (ITDs) in the *FLT3* receptor tyrosine kinase gene have long been known to confer a poor prognosis to acute myeloid leukemia (AML) patients. Now, specific structural features of the ITD are also suggested to alter patient outcome, including sensitivity to targeted therapies, prompting their evaluation in therapeutic algorithms.

(54 words)

## Commentary

In this issue of *Clinical Cancer Research*, Schwartz and colleagues show that structural features of *FLT3*-internal tandem duplications (ITDs) mutations, a highly recurrent mutation in acute myeloid leukemia (AML), influence patient responses and overall outcome following both standard cytotoxic chemotherapy and targeted *FLT3* inhibitor therapy(1).

AML is a genetically heterogeneous disease and several subtypes, characterized by different biological and prognostic features, have been described based on their mutational patterns and the presence of gross chromosomal alterations (2). Mutations affecting genes involved in signaling pathways are amongst the most frequently found in AML. In particular, mutations in the type-III receptor tyrosine kinase (TK) *FLT3* are present in about 30% of AML patients. *FLT3* mutations are mostly secondary to an internal tandem duplication (*FLT3<sup>ITD</sup>*) of the juxtamembrane domain which abrogates its negative regulatory function, resulting in constitutive activation of the receptor's TK activity, and predict for an increased relapse rate following standard therapies and a poor prognosis(3).

*FLT3<sup>ITD</sup>* mutations activate survival and proliferative signaling pathways, including phosphatidylinositol3-kinase (PI3K)/AKT, mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and signal transducer and activator of transcription 5 (STAT5) thus explaining the hyperproliferative and aggressive phenotype of AML carrying these mutations. Quantification of variant allele frequency in patient DNA samples, genetic assessment at diagnosis and relapse and studies in animal models have respectively shown

that *FLT3<sup>ITD</sup>* occur later in leukemia evolution and are not sufficient to produce an AML phenotype without collaborating mutations. Nevertheless, preclinical studies demonstrate that AML cells are oncogenically addicted to *FLT3<sup>ITD</sup>* thus suggesting its role as a therapeutic target(4). This has been also confirmed by the results of several recent phase 2 and 3 clinical trials that have demonstrated a survival benefit for patients treated with FLT3 TK inhibitors (TKI)(3). However, despite our improved understanding of the role played by *FLT3<sup>ITD</sup>* mutations in AML and the rational design of targeted inhibitors of their TK activity, the overall outcome of AML patients carrying *FLT3<sup>ITD</sup>* mutations remains poor and somewhat variable, with average overall response rates of less than 50% even following treatment with targeted FLT3 TKI. Several mechanisms have been shown to alter responses to FLT3 TKI including mutations in the FLT3 TK domain, cellular adaptive responses and disease features inherent to individual patients, such as *FLT3<sup>ITD</sup>* mutational burden and the presence of co-occurring mutations(2-4).

The manuscript from Schwartz et al. provides a novel tool to further classify patients response to both standard cytotoxic and novel FLT3 TKI therapies based on the ITD structural features of *FLT3<sup>ITD</sup>* mutations. The authors developed a novel, publicly available, bioinformatic algorithm (HeatITup) which is able to detect the presence of *FLT3<sup>ITD</sup>* mutations from DNA next-generation sequencing data from AML patients at diagnosis with better accuracy than other commonly used algorithms, validating their findings using both sequencing data from their own independent patient cohort and the TCGA AML dataset. Moreover, they show that HeatITup is capable of defining in fine details the structural features of ITD mutations in each patient. These include not only length of the duplicated section but also nucleotide composition including spacer regions and exogenous sequence within the insertion, as well as potentially relevant point mutations within the ITDs. Using HeatITup, the authors then divide *FLT3<sup>ITD</sup>* mutations into "typical" or "atypical" subtypes, based respectively on the absence or the presence of nucleotides exogenous to the wildtype *FLT3* locus within the mutated region. Interestingly, they demonstrate that typical and atypical mutations tend to cluster within each

patient, i.e. patient harbouring multiple ITD clones tend to have mostly either typical or atypical clones. This suggests that these 2 types of mutations might represent different biological entities and/or represent the consequences of different mutational exposures or processes. Partially supporting this notion, they then show that patient carrying atypical *FLT3<sup>ITD</sup>* mutations have lower survival rates when treated with both standard cytotoxic chemotherapy and FLT3 TKI therapy, within both their patient cohort and validated in the TCGA cohort.

Although *FLT3<sup>ITD</sup>* allele burden, reflective of the size of the FLT3-mutated clone and or uniparental disomy for chromosome 13q, has been implicated in the prognosis of patients treated with standard chemotherapy, the role of duplication length is less well understood as there have been conflicting reports regarding a link between overall survival and ITD length(5). The work by Schwartz and colleagues adds to our knowledge on the role of structural features of *FLT3<sup>ITD</sup>* mutations by showing that, beside length, other structural features of the ITDs may have an impact on patient outcome and that this might also hold true in the era of novel targeted therapy with FLT3 TKI. These findings will need to be validated in other independent datasets and the software the authors have developed will provide an accessible computational algorithm to enable these studies. If confirmed in larger studies, these results will also support the concept that characterization of *FLT3<sup>ITD</sup>* complexity, beside their detection, should be used to inform clinical decision-making and in the therapeutic algorithm for AML patients carrying *FLT3<sup>ITD</sup>* mutations.

Some of the observations from the author's work, if further confirmed, also raise interesting biological questions that, if confirmed, should be specifically addressed in experimental models of *FLT3<sup>ITD</sup>* leukemia. In particular, it would be interesting to understand how the 2 classes of ITD mutations confer a different response/sensitivity to treatment. Important questions could include; Does the type of ITD alter the native activity of mutated FLT3 and are typical and atypical ITDs different in the intensity of their signalling and FLT3 activity? Alternatively, as the 2 classes predict response to both standard cytotoxic and FLT3 TKI therapy, is the differential sensitivity to therapy independent of FLT3 activity and reflect other

processes such as increased/altered mutagenesis? Moreover, given that typical and atypical mutations tend to cluster within each patient, do these 2 types of mutations represent the consequences of different mutational exposures or processes? Interestingly, the author's data show that the pattern and number of co-occurring mutations is not different between the 2 classes of ITDs within their cohort, which seems to suggest similar rates of mutation. Nevertheless, further validation in bigger cohorts and a more in depth analysis of their biology might help to clarify if patients carrying each subtype of mutations show differential response to DNA damage and highlight the mechanisms behind their almost complete mutual exclusivity.

In conclusion, the manuscript by Schwartz represents another incremental step towards implementing precision medicine for AML patients carrying FLT3<sup>ITD</sup> mutations. Their HeatITup algorithm will be a valuable, easy-to-use and publicly available tool to stratify patients according to the structural features of their ITD mutations. It is also conceivable that this algorithm could be used to generically detect and classify ITD mutations reported in various genes for other subtypes of leukemia and solid cancers. Future studies will help to further validate these findings, clarify the features modulating the response to FLT3 TKI and standard chemotherapy in AML patients and understand their underlying biological mechanisms.

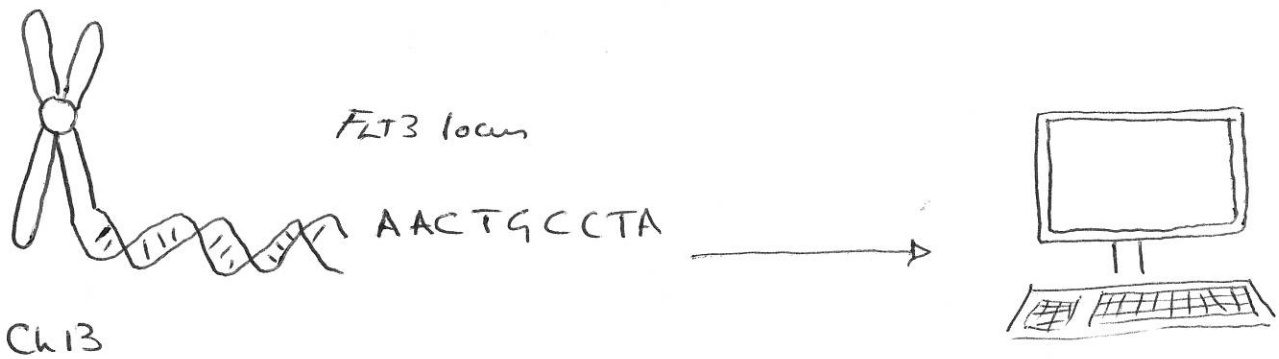
1142 words excluding abstract.

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## Figure Legend

Sequencing data from AML patients DNA can be analysed using the authors HeatITup algorithm to detect FLT3<sup>ITD</sup> mutations and classify them, based on the ITD structural features, in typical or atypical. This classification could then be used to inform patient prognosis and design more effective therapeutic algorithm for AML patients carrying *FLT3*<sup>ITD</sup> mutations.

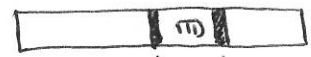


"Typical" ITD



vs

"Atypical" ITD



Exogenous  
Sequences

- Patient outcomes.
- Response to therapy.
- ? FLT3 signalling.
- ? Mutational processes.