Elrig Drug Discovery
Abstract (3987/4000 characters)

Authors: Jarno Kivioja[1], Disha Malani[1], Ashwini Kumar[1], Olli Kallioniemi[1], Kimmo Porkka[3], Caroline A. Heckman[1]

[1]Institute for Molecular Medicine Finland - FIMM, Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland;
[2]Hematology Research Unit Helsinki, University of Helsinki, and Helsinki University Central Hospital Comprehensive Cancer Centre, Department of Hematology, Helsinki, Finland

FLT3-ITD mutational burden predicts FLT3 inhibitor response in acute myeloid leukemia

Background
FLT3 (Fms-like tyrosine kinase 3) internal tandem duplication (*FLT3*-ITD) is a frequently observed and prognostically significant aberration in acute myeloid leukemia (AML) with incidence of roughly 25%. Recently two FLT3 inhibitors, namely gilteritinib and midostaurin, were approved for treatment of FLT3-mutated AML patients in combination with chemotherapy. Regardless of the confirmed survival benefit of FLT3 inhibitors, many *FLT3*-ITD$^+$ patients are unresponsive and most patients eventually develop drug resistance. Here, our aim was to determine whether *FLT3*-ITD mutational load or length of ITD predicts *ex vivo* response to FLT3 inhibitors in AML.

Materials and Methods
Fragment analysis was performed on 119 samples from 87 adult AML patients using 10 ng of genomic DNA with primers 5'-6-FAM GCAATTTAGGTATGAAAGCCAGC-3' and 5'-CTTTCA GCATTTTGACGGCAACC-3'. PCR-products were diluted with MQ-water (1:100-200) and 2 µl of each dilution was added to a 10 µl reaction mixture containing Hi-Di and a size standard. Samples were screened with ABI3730xl instrument (Illumina) and analyzed using GeneMapper software. Ficoll-separated bone marrow mononuclear cells (BM MNCs) were available for drug testing from 56 patients (74 samples). Drug testing was performed by seeding primary BM MNCs (10,000/well) in mononuclear cell medium (MCM) on 384-well plates containing ten FLT3 inhibitors in five concentrations (0,1 – 1,000 nM). The drug plates were kept in a humidified incubator at +37°C for 72 hours. After drug exposure, cell viabilities were measured with CellTiter-Glo® reagent (Promega) using a PHERAstar® FS microplate reader (BMG Labtech). Luminescence from each well was normalized to signal coming from positive (BzCl) and negative (DMSO) control wells. The normalization was necessary to produce dose-response curves based on normalized survival. A drug sensitivity score for each FLT3 inhibitor was calculated from the area under the curves as described (Yadav et al, 2013). Statistical analyses were performed in GraphPad Prism software.

Results
By fragment analysis, we identified *FLT3*-ITD from 51 samples collected from 38 patients. The median allelic burden of *FLT3*-ITD in the positive samples was 0.338 (range 0.015 – 1.0), while ITD lengths varied between 17 and 213 nucleotides (median 44.5). We initially assessed FLT3 inhibitor responses between *FLT3*-ITD$^+$ (n = 23) and *FLT3*-ITD$^-$ (n = 46) AML patient samples. Five *FLT3*-ITD$^-$ samples from the available data were excluded due to presence of *FLT3*-TKD mutation. We found that *ex vivo* response to all FLT3 inhibitors was significantly higher in the *FLT3*-ITD$^+$ patients compared to *FLT3*-ITD$^-$ samples with *P*-values ranging from 0.01 (lestaurtinib) to < 0.0001 (e.g. quizartinib, sorafenib). By assessing whether *FLT3*-ITD length affects sensitivity to FLT3 inhibitors, we found no correlation for any of the FLT3 inhibitors. Intriguingly, by analyzing the role of *FLT3*-ITD mutational burden on drug response, we discovered strong correlations. The correlation coefficient R-values were the highest for second-generation FLT3 inhibitors, namely crenolanib (R = 0.7622) and quizartinib (R = 0.7109), and the lowest for the less-specific first-generation FLT3 inhibitors such as ponatinib (R = 0.5306) and lestaurtinib (R = 0.3920). In our study, samples with higher *FLT3*-ITD mutational load also tended to have higher blast counts (R = 0.4864), while *FLT3*-ITD length showed no correlation with the amount of blast cells.

Conclusions
Our results suggest that *FLT3*-ITD mutational load could be an important parameter for deciding whether to administer *FLT3*-ITD$^+$ AML patients a specific or non-specific FLT3 inhibitor in combination with chemotherapy. The data implies that *FLT3*-ITD$^+$ patients with high *FLT3*-ITD mutational burden could benefit more from specific FLT3 inhibitors, while patients with low amounts of FLT3-ITD could respond better to non-specific FLT3 inhibitors targeting several receptor tyrosine kinases.