

Dasatinib response in acute myeloid leukemia is correlated with *FLT3/ITD*, *PTPN11* mutations and a unique gene expression signature

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Supplementary Methods

Drugs library

The 46 drugs cover a broad range of pathways and mechanisms of action. A large group of drugs which we tested are FLT3 targeted tyrosine kinase inhibitors, a common mutation with adverse prognosis in AML. Among them we examined some multi targeted tyrosine kinase (midosturin, dasatinib, SGI-1776 and pexidartinib) and second generation FLT3 inhibitors that are more specific and potent (quizartinib, G-749, crenolanib). We also examined the in vitro sensitivity of tyrosine kinase inhibitors of active signaling transduction pathways in AML like PI3K, mTOR, c-Kit, MEK, PIM, JAK1/2, hedgehog and PLK.

Other group of drugs tested is epigenetic modifiers: bromodomain and extra terminal protein (BET inhibitors), histone deacetylase (HDAC) inhibitor, DNA methyltransferase inhibitor (HMA), lysine specific demethylase 1 (LSD-1), histone methyltransferase (*DOT1L*) inhibitor and the mutated isocitrate dehydrogenase type 1 (*IDH1*) inhibitor. In addition, we examined the sensitivity in vitro of primary AML cells to chemotherapy, proteasome inhibitors, anti-apoptotic proteins inhibitors, and cell cycle inhibitors of *MDM2*, polo like kinase (*PLK*), cyclin dependent kinase (*CDK6*), and aurora kinase B.

Exome sequencing

Using this technique the DNA sequence of all protein-coding genes in a genome was obtained. The subset of DNA that encodes proteins (known as exoms), was selected and sequenced using high throughput DNA sequencing technology. In our unit, the Exome enrichment was performed using the Agilent SureSelect Human All Exon 50-Mb Kit, covering ~23,000 genes. Captured DNA libraries were sequenced with Illumina Hi-SEQ 2500 in a 100 paired end run to a coverage of > 150x (3/4 lane ~ 60 Million reads). This deep coverage data is sufficient to detect both SNPs and somatic mutations with high sensitivity. The data was analyzed using the state-of-the-art pipeline from the Broad institute for variant calling (GATK, MuTect2). In total exome analysis was performed on 26 samples.

Reads for each sample were mapped independently using BWA (v-) with the standard parameters to the reference genome. Uniquely mapping rates was observed. Variant calling was performed for 62 tumor/normal paired samples using the novel MuTect2

software, which is a somatic SNP and INDEL caller, from the Genome Analysis Toolkit (GATK) toolset (1). Variant effect predictor (VEP) was used for the annotation (2). 54 genes were defined as AML's drivers (3). All Variants related to these genes were extracted from the current exome analysis.

References:

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2. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome Biol.* 2016;17(1):122
3. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med.* 2016;374(23):2209-2221

Supplementary legends:

Supplementary Figure 1

The drug screening assay template with 46 drugs and chemical probes were distributed row-wise across 2 x 384-well plates, where each column represents a different compound. Sequentially reducing doses of compound were pre-plated before addition of cell culture to give final concentration depicted on left.

Supplementary Figure 2

Expression of 37 genes by mRNA sequencing in 29 primary AML samples from our study (A) and in 195 AML samples from the Beat AML cohort (B). Unsupervised hierarchical clustering shows in both studies a group of AML patient samples with overexpression of *FGR*, *HCK*, *LYN*, *PTK6*, *CSK*, *GAK* and *EPHB2* genes (marked in the black square) which was significantly more common in the dasatinib sensitive samples.

Supplementary Figure 3

Expression level of dasatinib target genes comparing between dasatinib responders and non-responders.

Supplementary Table 1

Samples from AML patients who achieved complete remission after induction chemotherapy were included. Patient's clinical characteristics are presented in Supplementary Table 1. For some of the cases a paired relapse sample was available for analysis. APL samples were excluded. Altogether 19 samples from diagnosis and 10 samples from relapse were included (out of them 7 were paired diagnosis and relapse samples). Patient gender, age at diagnosis and karyotype analysis and the origin of the sample from diagnosis and /or relapse are shown. Mutations status is defined as FLT3 internal tandem duplication (ITD, tyrosine kinase domain (TKD) with/ without nucleophosmin-1 (NPM1); N/A, not available. NK, normal karyotype.

Supplementary Table 2

Drugs list, class, their mechanism of activity and use in AML therapy is shown.

Supplementary Table 3

The samples number from the Beat AML study, the IC50 for dasatinib sensitivity in vitro and the classification to responder or not are presented. In addition, samples which exome and RNA sequencing was available where marked as 1 as compared to 0 for the samples with no available data.

Supplementary Table 4

Differentially expressed of dasatinib targeted genes between dasatinib responders and non-responders from the Beat AML study is shown. The selection of dasatinib targeted genes was based on the KinMap.

Supplementary Table 5

Differential gene expression analysis of responders and non-responders to dasatinib in our cohort

Supplementary Table 6

Differential gene expression analysis of responders and non-responders to dasatinib in the Beat AML study

Supplementary Table 7

The IC50 for dasatinib in samples from the Beat AML study carrying mutations in *FLT3/ITD* (positive) and wild type (negative) is shown.

Supplementary Table 8

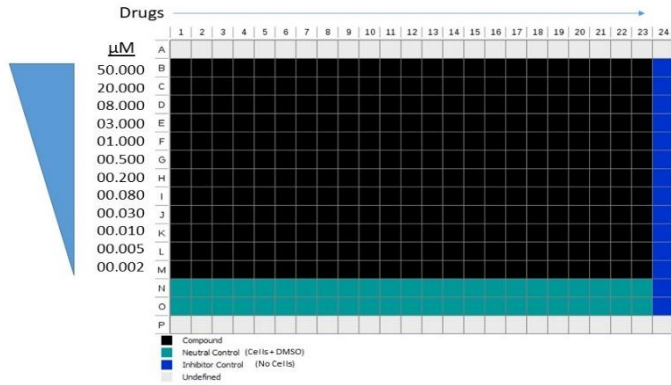
The IC50 for dasatinib in samples from the Beat AML study carrying mutations in *PTPN11* and wild type (WT) is shown.

Supplementary Table 9

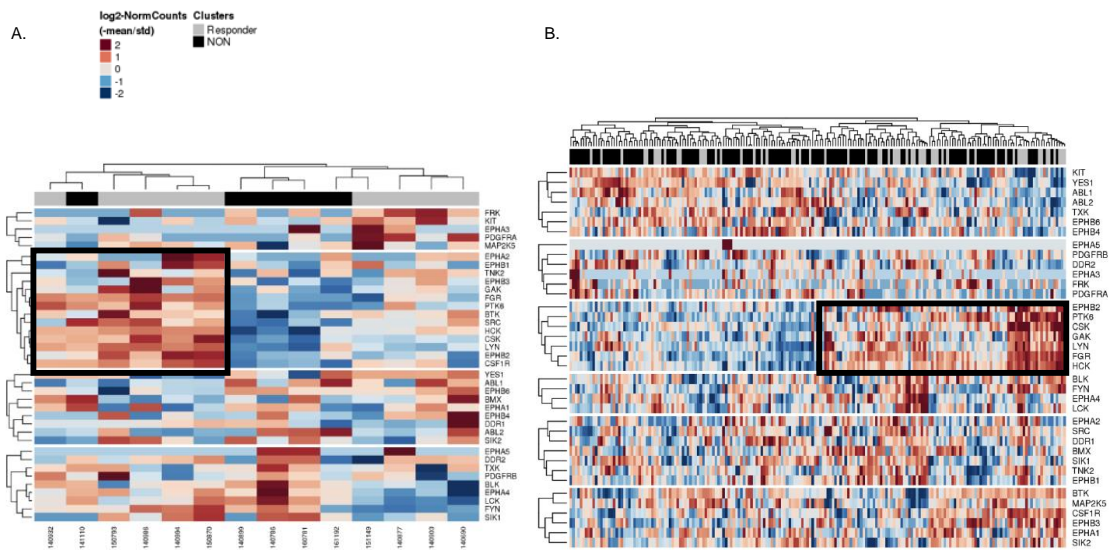
The IC50 for dasatinib in samples from the Beat AML study carrying mutations in *TP53* (mut) and wild type (WT) is shown.

Supplementary Table 10

The pathway analysis of intersecting upregulated genes INCPM and Beat AML cohorts



Supplementary Figure 1



Supplementary Figure 2

