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

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Haematologica 2020 [Epub ahead of print]

Citation: Sigal Tavor, Tali Shalit, Noa Chapal Ilani, Yoni Moskovitz, Nir Livnat, Yoram Groner, Haim Barr, Mark D. Minden, Alexander Plotnikov, Michael W. Deininger, Nathali Kaushansky, and Liran I. Shlush. Dasatinib response in acute myeloid leukemia is correlated with FLT3/ITD, PTPN11 mutations and a unique gene expression signature.
Haematologica. 2020; 105:xxx

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Dasatinib response in AML is correlated with FLT3/ITD, PTPN11 mutations and a unique gene expression signature

Running Title: Dasatinib response in AML

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Abstract

Novel targeted therapies demonstrate improved survival in specific subgroups (defined by genetic variants) of acute myeloid leukemia (AML) patients, validating the paradigm of molecularly targeted therapy. However, identifying correlations between AML molecular attributes and effective therapies is challenging. Recent advances in high-throughput in vitro drug sensitivity screening applied to primary AML blasts were used to uncover such correlations; however, these methods cannot predict the response of leukemic stem cells (LSCs). Our study aimed to predict in vitro response to targeted therapies, based on molecular markers, with subsequent validation in LSCs. We performed ex vivo sensitivity screening to 46 drugs on 29 primary AML samples at diagnosis or relapse. Using unsupervised hierarchical clustering analysis, we identified group with sensitivity to several tyrosine kinase inhibitors (TKIs), including the multi-TKI, dasatinib, and searched for correlations between dasatinib response, exome sequencing and gene expression from our dataset and from the Beat AML dataset. Unsupervised hierarchical clustering analysis of gene expression resulted in clustering of dasatinib responders and non-responders. In vitro response to dasatinib could be predicted based on gene expression (AUC=0.78). Furthermore, mutations in FLT3/ITD and PTPN11 were enriched in the dasatinib sensitive samples as opposed to mutations in TP53 which were enriched in resistant samples. Based on these results, we selected FLT3/ITD AML samples and injected them to NSG-SGM3 mice. Our results demonstrate that in a subgroup of FLT3/ITD AML (4 out of 9) dasatinib significantly inhibits LSC engraftment.

In summary we show that dasatinib has an anti-leukemic effect both on bulk blasts and, more importantly, LSCs from a subset of AML patients that can be identified based on mutational and expression profiles. Our data provide a rational basis for clinical trials of dasatinib in a molecularly selected subset of AML patients.
Introduction

AML is an aggressive myeloid neoplasm with complex and heterogeneous genetics that influence prognosis and treatment response. Furthermore, AML is a multi-stage disease with preleukemic, leukemic and late stages. Identification of AML-inducing mutations is required for accurate diagnosis and treatment to tailor therapy according to individual patients’ genetic profile. Recent advances in AML targeted therapy based on driver mutations have improved overall survival (2, 3). Increased molecular understanding of AML pathophysiology provided new opportunities to target specific mutants, such as $FLT3/ITD$ (FMS-like tyrosine kinase-3, internal tandem duplications) and $IDH1/2$ (5, 6). Moreover, the BCL2 inhibitor, venetoclax, has increased activity in $NPM1c$ and $RAD21$ mutated patients (7), and in combination with hypomethylating agents (HMAs) targets leukemia stem cells (LSCs) (8). While these novel therapies can achieve prolonged remissions, most patients eventually relapse. Our recent studies suggest that the origins of AML relapse are heterogeneous, but relapsing clones invariably exhibit stem cell properties (9). Relapse usually originates from leukemic sub clones that were present before therapy, and were either selected due to resistance mechanisms (9, 10) or gained competitiveness through further evolution, possibly due to chemotherapy exposure (11). Targeted therapies can fail in preventing relapse by addressing one leukemic cell clone, while allowing other clones to expand and cause relapse (12). In order to prevent relapse, it is critical to eradicate LSCs in the early stages of treatment (induction/consolidation) before they expand and acquire additional resistance mechanisms. It has been suggested that combination therapies that simultaneously target multiple leukemic sub clones may overcome AML heterogeneity. However, combination therapy approaches are limited by tolerability, particularly in older individuals, and it is important to maximize efficacy and minimize toxicity by selecting the right drug for the right set of patients. Given the multitude of therapy options, it is essential to design clinical trials based on molecular markers that select for those patients likely to benefit.

Our latest studies demonstrate that in certain AML cases where relapse originated from primitive cells (ROp) (9) the cells from the time of diagnosis capable of leukemic engraftment in immune deficient mice were identical to those that caused relapse. In these patients the bulk of cells are responsive to chemotherapy, but rare LSCs that expand in xenografts drive of relapse. Accordingly, drugs which inhibit the engrafting clones should be useful in preventing relapse, but screening a large number
of drugs in a large number of samples in xenografts is not feasible. An alternative approach is to screen for drugs with known mechanistic effects against large and heterogeneous AML cohorts \textit{in vitro}, which allows for high throughput screening of many drugs to establish correlations between response and molecular attributes of AML. Disadvantages include the lack of microenvironment and immune system and the short duration of drug exposure, which reflects the inability of AML cells to grow \textit{in vitro}. Short-term \textit{in vitro} drug sensitivity assays became feasible in recent years by mirroring normal hematopoietic stem and progenitor cell - culture conditions. So far \textit{in vitro} drug testing of primary AML samples focused on predicting clinical outcome (13, 14), and, in some cases, guiding individualized therapy (15). Such personalized therapy is particularly important for relapsed/refractory disease (16). \textit{In vitro} drug studies identified biomarkers of response to specific drugs (17) and clusters of AML samples with similar response profiles (18). In the current study, we studied \textit{in vitro} drug sensitivity of primary AML samples from patients who achieved complete remission (CR) and compared our results to the Beat AML study. Our global aim was to identify therapies and biomarkers, which could predict which drugs might be added to induction therapy to prevent relapse in specific subtypes of AML and then validate the results \textit{in vivo}. We discovered that samples with \textit{in vitro} sensitivity to dasatinib (a multi kinase inhibitor) had a specific \textit{in vitro} drug sensitivity pattern, gene expression signature, and were enriched for \textit{FLT3}/ITD and \textit{PTPN11} mutations. Xenograft studies confirmed that dasatinib targets LSCs \textit{in vivo}.

\textbf{Methods}

\textbf{Primary AML cells}

Frozen mononuclear cells (MNC) from bone marrow or peripheral blood of AML patients at diagnosis and relapse when available were used. Inclusion criteria for sample selection were achievement of CR. Patients with low peripheral blood blast count and acute promyelocytic leukemia (APL) were excluded (Supplementary Table 1). Ethics approval: UHN 01-0573

\textbf{Drug library}

We selected FDA-approved cancer drugs with demonstrated clinical and or preclinical efficacy in leukemia and in other cancers. The 46 drugs and their mechanisms of action are detailed in Supplementary Table 2.
Drug screening technology

Cells were thawed, washed, counted and suspended in RPMI supplemented with 10% FCS and human cytokines: stem cell factor (CSF) 100 ng/ml, interleukin 3 (IL-3) 10 ng/ml, interleukin 6 (IL-6) 20 ng/ml and thrombopoietin (TPO) 10 ng/ml. Sensitivity profiling was done by dose-response over several log ranges (Supplementary Figure 1). Assay-ready plates in 384 well format was arrayed in 12 concentrations for each compound (2nM -50μM), using a Labcyte Echo 555 acoustic dispenser, and frozen until use. Patient samples were dispensed at approximately 1000 cells per well and cultured for two days. Viable cells were quantified using Cell Titer Glo reagent (Promega Madison, WI). Data was normalized using Genedata Screener software where DMSO treated cells correspond to 100% viability and samples without cells correspond to 0% viability. Normalized data was loaded to CDD Vault software to calculate IC50, minimum and maximum responses for each drug and patient. We compared our in vitro response to dasatinib with the results of the Beat AML drug sensitivity and resistance testing (DSRT). In the Beat AML study in vitro cultures were done without cytokines (17).

RNA and exome sequencing

The workflow for RNA sequencing was performed as previously described (9) and the methods for RNA sequencing, exome sequencing, and gene expression and mutation analysis are detailed in the supplementary methods. For pathway analysis we used enrichr (19, 20).

Prediction of response to dasatinib

Dasatinib responders were defined in our cohort and in the Beat AML cohort as IC50 <0.01, and dasatinib non-responders in both cohorts as IC50>0.8 (Supplementary Table 3). To identify predictors of response to dasatinib we initially selected genes based on their differential expression (DE) between dasatinib responders and non-responders. Furthermore, other gene sets were selected based on pathway analysis of the DE genes and genes known to be targets of dasatinib as reported by the KinMap (Supplementary Table 4, genes with Kd(nM)<7 (37 genes)). To refine feature selection, we tested which dasatinib target genes were differentially expressed between dasatinib responders and non-responders from the Beat AML study (Supplementary Figure 3, Supplementary Table 4). Various machine learning models were tested all with cross validation of 10-fold, on the Beat AML data only. The best prediction model was k Nearest Neighbor (KNN) algorithm with cosine similarity.
This model was validated on the Israel National Center for Personalized Medicine (INCPM) gene expression data. (Matlab statistical tool box).

**In Vivo dasatinib treatment**

SGM3 mice were injected intra-femoral with 1e6 CD3 depleted AML human samples. From day 35 post-injection mice were treated orally with 50 mg/kg dasatinib for 21 days and scarified on day 56. FACs analysis was performed. BM cell suspensions were analyzed for expression of human CD45 (BV521, clone HL30), CD34 (APCcy7, clone 581), CD38 (PE cy7, clone303516), CD15 (BV421, clone W6D3) Biolegend products, CD33 (APC), CD3 (FITC) and CD19 (PE) markers BD products. Human engraftment was defined as >0.1% of cells positive for human CD45 at the time of sacrifice. Comparison of percent engraftment of human cells in drug-versus control-treated mice was performed using the Wilcoxon test. The IACUC of the Weizmann Institute approved the experiments, which were performed in accordance with its relevant guidelines and regulations (11790319-2).

**Statistical analysis**

Heatmap and clustering of drug sensitivity analysis was performed with R package ComplexHeatmap. Difference between the defined groups were validated by Statistical analysis in figures 1A, and Wilcoxon rank-sum test in figures 5 and 6. Enrichment analyses were performed using Fisher’s exact test. Differential expression (DE) genes analysis was performed using DESeq2 package (1.10.1) (21) with the betaPrior, coks Cutoff and independent Filtering parameters set to False. Raw P values were adjusted for multiple testing using the procedure of Benjamini and Hochberg. DE genes, were determined by a p-adj of < 0.05 and absolute fold changes > 2 and max raw counts > 30. (Figure 2, Supplementary Table 5 and Table 6). Overlap between DE genes in the Beat AML and INCPM was tested by the hypergeometric distribution by the following R function sum (dhyper(t:b, a, n - a, b)). List a contains the DE genes in the Beat AML dataset, and b the DE genes in INCPM. The total number of expressed genes in both datasets was n=20055. The intersection between a and b is t (Figure 3A).

**Results**

**Classification of AML samples based on drug response, and mutation profile**

The overall goal of the current study was to identify drugs that can be safely given at diagnosis and target LSCs. To achieve this goal, we first tried to identify drugs which were more effective at relapse compared to diagnosis. Such drugs would presumably
target LSCs which are enriched at relapse. Comparing the DSRT between AML diagnosis and relapse paired samples we found that for the majority of tested compounds the IC50 was higher in the relapse samples (Figure 1A), however statistically insignificant in most cases. Clinically relevant drugs that are being used to treat patients at relapse (etoposide (22) midostaurin (23) and fludarabine (24) were significantly less effective at relapse Figure 1A). Next, we took an unbiased approach to identify subgroups of AMLs sensitive to specific drugs to identify patients likely to benefit from specific targeted therapies. We screened 29 primary AML samples for DSRT using a test set of 46 drugs. Five compounds (BEZ235, lenalidomide, visomodegib, EPZ-5676 and AG-120) were excluded from the analysis due to low variability in the response among the different samples (almost all samples were resistant to these drugs). Unsupervised hierarchical clustering analysis of IC50 values demonstrated two distinct drug response patterns (Figure 1B). The first group displayed high sensitivity to TKIs (dasatinib, MEK inhibitors, PI3K inhibitor (BKM120) pim inhibitor, ruxolitinib, mTORC1/2 inhibitor (INK128), quizartinib, midostaurin), BET inhibitors (OTX015, JQ1) and several other drugs. This group was termed sensitive. The second group was resistant to most compounds. The "resistant" group displayed heterogenous sensitivity to BCL2 inhibitors (ABT-737 and venetoclax), purine analogues (clofarabine, fludarabine,) and the hypomethylating agent decitabine. Interestingly, the in vitro sensitivity to BCL2 inhibitors (ABT-737 and venetoclax) was not clustered in a specific group, consistent with clinical observations (Figure 1B) (8, 25). To define the molecular pathways underlying the drug sensitivity of the clustering groups, we analyzed the somatic mutations (exome sequencing) and gene expression of the AML samples. We focused our genetic analysis on recurrently mutated genes in AML (26). Mutations in FLT3/ITD were observed in 7 out of 17 patients in the "sensitive" group and none out of 12 in the "resistant" group (p=0.023). No other significant enrichment was identified in the current dataset.

**Gene expression and mutation profile of dasatinib sensitive AML cells**

Analysis of the in vitro DSRT assay, identified a subgroup of AML samples sensitive to a broad range of TKIs (Figure 1B). Among the TKIs, dasatinib sensitivity was different between the two groups. In particular, in the sensitive group 52% (9 out of 17) of the AML samples displayed sensitivity to dasatinib (defined as a 10 fold difference in IC50 between sensitive and non-sensitive) whereas in the resistant none
of the samples showed in vitro sensitivity to dasatinib (p=0.003) (Figure 1B). Based on these results we focused our analysis on dasatinib which has been safely for many years as monotherapy or in combination with chemotherapy for Philadelphia positive (Ph+) leukemia. Next, we set to define those molecular features that could predict which AML samples would be sensitive to dasatinib. As our cohort was small, we compared our results to those of the Beat AML study (17). We classified dasatinib responders (N=61) and non-responders (N=134) based on IC50 values (Supplementary Table 3). Other parameters including: age, gender, leukocyte number, time of sample (diagnosis /relapse), type of AML (de novo /transformed), karyotype and mutation profile were also tested for correlation with dasatinib response.

Differential gene expression analysis of responders and non-responders to dasatinib in our cohort identified 297 genes significantly overexpressed and 404 genes downregulated in the sensitive group and (FDR<0.05) (Supplementary Table 5). In the Beat AML cohort, we identified 300 significantly upregulated and 720 downregulated genes in the dasatinib responder group as compared to the non-responder group (Supplementary Table 6). Unsupervised hierarchical clustering of all expressed genes enriched dasatinib responders in specific clusters both in our cohort (figure 2A) and in the Beat AML cohort (Figure 2B). Intersecting the upregulated and downregulated DE genes from the Beat AML and the INCPM cohorts resulted in a significant number of intersecting genes (P<e-23, P<e-21 respectively), (Figure 3A).

Pathway analysis of intersecting upregulated genes in both cohorts identified significant enrichment of genes that are co-expressed with dasatinib targets (CSF1R, SRC) (Figure 3B, Supplementary Table 10). On the other hand, the same pathway analysis of intersecting downregulated genes did not enrich for dasatinib targets. As we identified upregulation of genes that are co-expressed with dasatinib targets we next performed a focused analysis on the expression of actual dasatinib targets. We identified all known dasatinib targets and performed unsupervised hierarchical clustering on their gene expression in both our cohort and the Beat AML. In both cohorts a cluster enriched with dasatinib responders was identified. In the Beat AML cohort 13 out of 21 samples (59%) were responders in this cluster as opposed to 61 out of 195 (31.3%) responders in the entire Beat AML cohort (p=0.016) (Supplementary Figure 2). In both cohorts the dasatinib target cluster included three SRC family tyrosine kinases FGR, HCK and LYN as well as the PTK6, CSK, GAK
and EPHB2. Several of these genes were significantly overexpressed in dasatinib responders in the Beat AML cohort (Supplementary Figure 3).

Next, we used the data from the gene expression analysis to establish a prediction model identification of dasatinib responders \textit{in vitro} based on gene expression. For all experiments we used a cross validation approach, training the model only on the Beat AML data and validating results on the INCPM data. We tested the following genes sets from the Beat AML data for prediction: all genes, all DE genes, all upregulated genes, all downregulated genes, all dasatinib targets and DE dasatinib targets. The best prediction model with sensitivity of 0.85, specificity of 0.7 (AUC=0.78) (Figure 4A, B) was achieved when using the Beat AML downregulated genes. When testing the same model on the INCPM data the positive predictive value was 100% and false discovery rate was 60% (Figure 4C). This data provides evidence that gene expression can predict \textit{in vitro} response to dasatinib, however with limited accuracy.

As gene expression did not provide an ideal prediction for dasatinib response, we tested whether any other parameters from the Beat AML could predict response to dasatinib. We analyzed molecular and clinical data for enrichment in dasatinib responders. We found that samples carrying mutations in \textit{FLT3}/ITD ($p=0.011$) (Supplementary Table 7), \textit{IDH2} and \textit{PTPN11} ($p<0.05$) were enriched in dasatinib responders in the Beat AML cohort. The IC50 for dasatinib in carriers of \textit{FLT3}/ITD and \textit{PTPN11} was significantly lower compared to the IC50 of non-carriers ($p<0.0001$, $p=0.02$ respectively) (Figure 5A, B and Supplementary Table 8), whereas only mutations in \textit{p53} had significantly higher IC50 compared to WT ($p=0.0003$) (Figure 5C and Supplementary Table 9). Adding the mutations status to the gene expression prediction model did not improve accuracy.

Altogether, these data suggest molecular features (mutations and gene expression profile) identify a group of AML cases responsive to dasatinib \textit{in vitro}. Specifically, the fact the \textit{FLT3}/ITD mutant cases are enriched in the dasatinib responders in both Beat AML and IBCPM cohorts prompted us to test the \textit{in vivo} efficacy of dasatinib in this specific group of patients. As our ultimate goal was to test whether \textit{in vitro} response to dasatinib would predict \textit{in vivo} AML response, we chose to focus our \textit{in vivo} dasatinib response studies on samples known to carry \textit{FLT3}/ITD. The focus on \textit{FLT3}/ITD stems also from the fact that \textit{FLT3}/ITD AML samples are known to be good AML engrafters (27).
reduces LSCs in mice engrafted with FLT3 ITD mutant AML

We injected NSG mice (N=5-10/sample) with cells from 9 patients with FLT3/ITD AML. On day 35 we randomized to dasatinib or carrier control. Following 3 weeks of treatment we measured human engraftment in treated and control groups (Figure 6). FACS of BM demonstrated leukemic engraftment (CD33+>90% of human CD45+ cells) in mice from both groups. For 4 out of 9 samples, engraftment was significantly reduced in the dasatinib-treated group. This result suggests that dasatinib has anti LSC activity in a subset of FLT3/ITD positive AML cases.

Discussion

We provide evidence that a subset of FLT3/ITD+ AML cases and AMLs with a unique gene expression pattern are sensitive to dasatinib in vitro and that this sensitivity translates into sensitivity in vivo, at least in FLT3/ITD positive cases. We initially discovered sensitivity to dasatinib using DSRT and validated the DSRT results based on the Beat AML study. DSRTs can only measure toxicity to bulk blasts cells and cannot detect effects on differentiation and LSCs. Accordingly, we tested the response of FLT3/ITD+ primary AML samples to dasatinib in a xenograft model and confirmed response in vivo.

FLT3/ITD is one of the most common mutations in AML, and is associated with high risk of relapse and mortality (28, 29, 30, 31). Recently the introduction of FLT3 TKIs has improved overall survival (32, 33), however not all FLT3/ITD+ patients respond to FLT3 inhibitors, whereas some FLT3/ITD- patients do (34). We show that FLT3/ITD AML cells are sensitive to dasatinib both in vitro and in xenograft models. FLT3/ITD results in constitutive activation of FLT3 and activates several signal transduction pathways such as RAS-MAPK, PI3K, JAK-STAT and SRC. This broad signal transduction activation might explain the heterogeneity observed in the response to FLT3 inhibitors. The currently standard of care for patients with FLT3/ITD is midostaurin combined with chemotherapy. However, in the RATIFY study only 46.4% of patients achieving CR after midostaurin remained disease free after 4 years with an ongoing risk of relapse, especially during the first year after treatment (23). The high failure rate even following combination therapy indicates that additional therapy is needed upfront. Previous studies by Weisberg et al
demonstrated synergist effects for the combination of midostaurin and dasatinib in FLT3/ITD mutated AML cell lines (35). Our in vivo data demonstrate that the anti-leukemic activity of dasatinib extends to FLT3/ITD LSCs, at least for a subset of patients and suggests that addition of dasatinib to current standard of care may be beneficial for FLT3/ITD AML patients.

Our results show that a unique gene expression signature correlates with dasatinib response. Consistent with mechanistic predictions dasatinib responders demonstrated overexpression of multiple dasatinib targets. SRC family kinase (SFK) genes, such as LYN, HCK (36) and FGR (which are all dasatinib targets) are overexpressed in dasatinib responders in the Beat AML dataset (Figure 3B, Supplementary Figure 3), and were shown to be overexpressed in AML-LSCs and to contribute to their survival and proliferation (38). Exposure to dasatinib treatment inhibited SFK phosphorylation in primitive and committed AML progenitors. In this study the combination of dasatinib and chemotherapy enhances LSC targeting by p53 signaling (38). Furthermore recent evidence suggests that FLT3 activates SFKs and they in turn regulate the activity of the RAS/ERK pathways (37). Altogether, our data suggest that dasatinib may be active not only in FLT3/ITD AMLs but also other AMLs sharing a similar dasatinib responsive gene expression signature. Similarly, we show that dasatinib may be effective in treating PTPN11 mutated AML, but likely not all PTPN11 mutated AMLs will respond to dasatinib and a larger cohort is needed to identify additional biomarkers. Mutations in PTPN11 are also found in 35% of patients with juvenile myelomonocytic leukemia (JMML), ALL, and in 4-7% of AML patients. PTPN11 encodes the Shp2 cytoplasmatic protein tyrosine phosphatase, and SHP2 mutations cause increased phosphatase activity that contributes to leukemogenesis by upregulating the RAS, JAK-STAT, and PI3K pathways, leading to aberrant proliferation of myeloid progenitors (39, 40). A recent study demonstrated that TNK2 activates PTPN11, furthermore, mutant myeloid leukemic cells were carrying PTPN11 activating mutations were sensitive to TNK inhibition by dasatinib in vitro (41). Future studies are needed to better define the subset of PTPN11 mutated AMLs likely to respond to dasatinib and test dasatinib activity in vivo.

Our results suggest that the addition of dasatinib to the current standard of care for FLT3/ITD AML (FLT3 inhibitor in combination with cytarabine/anthracycline) may benefit a subset of FLT3/ITD+ patients and should be tested in a clinical trial.
Similarly, patients ineligible for intensive induction therapy may benefit from combining dasatinib, FLT3 TKI, HMAs and venetoclax. Clearly the potential toxicity of such combinations needs to be taken into account. Another possible clinical trial would be the addition of dasatinib to standard of care in patients with \textit{PTPN11} mutated AML. A unique gene expression signature as detected by RNA-seq may predict response to dasatinib, suggesting that RNA-seq-based patient selection may be included in future clinical trials. Given dasatinib’s activity against LSCs, there is hope that addition of dasatinib during initial therapy will reduce the risk of subsequent relapse.
References


Figure legends

1A. In vitro drug resistance to most drugs is acquired after relapse. An average drug response of seven coupled of primary AML samples, comparing the IC50 between diagnosis and relapse samples of 41 drugs. Each dot represents the response for specific drug, calculated by the median IC50 ratio of diagnosis vs. relapse. B. Drug sensitivity and resistance testing (DSRT) of primary AML cells. Hierarchical clustering using Pearson’s dissimilarity and complete linkage was performed. Data was the log2 IC50 +0.001, standardized for each compound by reducing the mean. A DSRT for 41 drugs of clinical and preclinical use in AML shows two clustering groups of patients. The age, gender, the origin of the patient sample from diagnosis or relapse, the name of the drugs, the class of the drug, karyotype and mutation status for FLT3/ITD and NPM1 is demonstrated.

2. Transcriptome profiling of dasatinib responder samples. Gene expression analysis of whole transcriptome mRNA sequencing comparing dasatinib sensitive to non-sensitive AML samples. Differential express genes between the "responders", and "non-responders" showed in A. In order to extend the tested samples we also applied the same analysis to AML patient samples from the Beat AML dataset (B).

3. Analysis of differentially expressed genes between dasatinib responders and non-responders from INCPM and Beat AML datasets. (A) Intersecting of upregulated (upper panel) and downregulated (lower panel) differentially expressed genes from the INCPM and Beat AML cohorts. (B) Pathway analysis of intersecting upregulated genes in both cohorts identified significant enrichment of genes that are co-expressed with several dasatinib targets. In red are dasatinib targets (CSF1R, SRC, BLK) and * marks significant enrichment (FRD<0.05).

4. Prediction model to identify dasatinib responders based on gene expression. (A, B) k Nearest Neighbor (KNN) algorithm with cosine similarity was used tp predict dasatinib response. Best accuracy was achieved with 10 fold cross validation applied to the differentially downregulated genes from the Beat AML dataset. Accordingly, sensitivity of 0.85, specificity of 0.7 were achieved. (C) Validation of the KNN cosine prediction model on INCPM data.
5. Correlation between FLT3/ITD, PTPN11 and p53 mutations in AML samples to dasatinib response in vitro in the Beat AML study. Median dasatinib IC50 value in mutated FLT3/ITD (A) PTPN11 (B) or P53 (C) and wild type (WT).

6. Dasatinib delays development of human FLT3/ITD mutated AML in transplanted mice. SGM3 mice were treated with dasatinib for 3 weeks after transplantation of human cells from nine patients with FLT3/ITD AML. Percentage of human CD45+ cells in engrafted in murine bone marrow with /without dasatinib treatment is shown after staining with CD45, CD34, CD33, CD38, CD15, CD3 and CD19 for determine the myeloid lineage cells and analyzed by fluorescence-activated cell sorting immunostaining (A) (∗, P < 0.05 Wilcoxon rank sum test, ** P < 0.005 *** P < 0.0005). The mutation status of the AML samples was: 140005: FLT3/ITD+, FLT3/TKD-, NPM1+; 40094: NPM1+, FLT3/ITD+, 150809: NPM1−, FLT3/ITD+, 40034: NPM1−, FLT3/ITD+, 150279: FLT3/ITD+, FLT3/TKD−, NPM1+; 130695: NPM1+, FLT3/ITD+, FLT3/TKD−; 160436: NPM1+, FLT3/ITD+; 160406: FLT3/ITD+, NPM1+; 130607: FLT3/ITD+, NPM1+
Acknowledgments:

This research was supported by a grant from the Nancy and Stephen Grand Israel National Center for Personalized Medicine to H.B. This research was supported by the EU horizon 2020 grant project MAMLE ID: 714731, LLS Grant ID: RTF6005-19, and IMOS-712843 awarded to LIS. LIS is an incumbent of the Ruth and Louis Leland career development chair. N.K. is an incumbent of the Applebaum Foundation Research Fellow Chair. This research was also supported by the Sagol Institute for Longevity Research, the Barry and Eleanore Reznik Family Cancer Research Fund, Steven B. Rubenstein Research Fund for Leukemia and Other Blood Disorders, the Rising Tide Foundation and the Applebaum Foundation.

Competing interests

The authors declare no competing interests.

Author Contribution

S.T. designed the study, analyzed data and wrote the manuscript, T.S. analyzed data, N.C.I. analyzed data, Y.M. performed DNA and RNA sequencing experiments, N.L. performed real time PCR experiments, Y.G. designed the study, H.B. and A.P. performed *in vitro* drug screen, M.D.M. designed the study, N.K. designed the study, performed mice and tissue culture experiments, wrote the manuscript, L.I.S. designed the study, wrote the manuscript, and oversaw the research.
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:


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 on Excel file**
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 on Excel file**
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 on Excel file**
Differential gene expression analysis of responders and non-responders to dasatinib in our cohort

**Supplementary Table 6 on Excel file**
Differential gene expression analysis of responders and non-responders to dasatinib in the Beat AML study

**Supplementary Table 7 on Excel file**
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 on Excel file 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 on Excel file**
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 on Excel file**
The pathway analysis of intersecting upregulated genes INCPM and Beat AML cohorts
Supplementary Table 1

<table>
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<tr>
<th>Patients No</th>
<th>Age/Sex</th>
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<th>NPM1</th>
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### Supplementary Table 2

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