SUPPLEMENT - METHODS

RARA Biomarker Test

A blood-based retinoic acid receptor alpha (RARA) biomarker test was developed and received FDA Investigational Device Exemption (IDE) approval for patient screening prior to treatment of tamibarotene in this Phase 2 study (Vigil et al, ESH 2017; Abstract 8882). The test measures relative *RARA* mRNA expression levels against a panel of control genes with stable expression levels via real-time quantitative polymerase chain reaction (RT-qPCR) in CD34+ and/or CD117+ blasts isolated from peripheral blood mononuclear cell (PBMCs) and applies a predefined cutoff to determine whether any given patient sample is RARA-positive or RARA-negative (Figure 1). Prior preclinical data demonstrated a clear relationship between *RARA* mRNA level (measured by RNA-seq) and response to tamibarotene in RARA-high human cell lines and acute myeloid leukemia (AML) patient-derived xenograft (PDX) models (McKeown et al, Cancer Discov. 2017; doi: 10.1158/2159-8290). The cutoff of RARA positivity for the biomarker test was established by applying the biomarker test to bone marrow samples from the PDX models responsive to tamibarotene versus those that were non-responsive (McKeown et al, Cancer Discov. 2017; doi: 10.1158/2159-8290).

A blood sample (8 mL in BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate) was collected from the patient at screening and processed into PBMCs at the clinical site following

manufacturer's instructions. PBMCs were preserved with freezing media and shipped on dry ice to a central diagnostic laboratory. After thawing, blast cells were enriched with CD34 and CD117 antibody magnetic beads, followed with RNA extraction. RT-qPCR was applied and averaged Cq value of the *RARA* gene was normalized against the averaged Cq values for the control genes to yield the ΔCq value. When performing a RT-qPCR test, Cq value is the number of cycles it takes to differentiate the target sequence from background fluorescence. The pre-determined cutoff for RARA positivity was applied to the ΔCq value for each patient sample to generate the biomarker result of either "RARA-positive" or "RARA-negative," which was then reported to the site from the central laboratory (Vigil et al, ESH 2017; Abstract 8882).

Due to the PBMC isolation step and the subsequent enrichment of the blast cell population with CD34 and CD117 antibody magnetic beads, a RARA biomarker test result can be obtained in patients without detectable blasts in the peripheral blood based on a standard complete blood count test.

***Ex vivo* Differentiation Assay**

Peripheral blood samples for *ex vivo* differentiation assay were collected in 8 mL acid citrate dextrose (ACD) Solution A tubes and were performed at Notable Labs (Foster City, CA) using a fully automated platform, as previously published (Vigil et al, ESH 2017, Abstract 8882; Spinner et al, Bood Adv. 2020, doi: 10.1182/bloodadvances). Patient blood samples were treated for 72 hours *ex vivo* with either dimethyl sulfoxide (DMSO) as the vehicle control or 50 nM

tamibarotene single agent and assessed for blast viability and differentiation with high-throughput, multi-parametric fluorescence-activated cell sorting (FACS). The total blast population was defined by blast markers (e.g., CD33, CD34, and/or CD117). Viability of total blasts, normalized to vehicle control DMSO was used to evaluate cytotoxicity. Differentiation was evaluated by identifying blast cells that expressed one of the following phenotypic markers of differentiation: CD11b and CD11c, CD66b, CD14, and/or CD38 (high). A random forest model was trained using the difference in these markers between tamibarotene and DMSO treatment in each patient to predict RARA positivity versus negativity using 5-fold cross-validation. The cross-validated model prediction was referred to as a differentiation model score, and the p-value between RARA-positive and RARA-negative populations was derived from a two-tailed t-test.

RARA Biomarker Cutoff

During screening, as part of an exploratory analysis, additional blood samples were available from 26 RARA-positive and 46 RARA-negative patients screened by the biomarker test. These patients were enrolled in early cohorts of the study and had the diagnosis of either lower-risk myelodysplastic syndromes (MDS), relapsed/refractory (R/R) higher-risk (HR)-MDS, R/R AML, or newly diagnosed (ND) AML. Samples were evaluated for differentiation responses of blasts to tamibarotene *ex vivo* (Jurcic et al, ASH 2017, Abstract 2633).

A random forest machine learning model was used to predict RARA biomarker status using change in a patient's immunophenotyping after *ex vivo* tamibarotene treatment compared to the DMSO control.

Prognostic Significance of RARA Biomarker

RNA-seq datasets were evaluated from The Cancer Genome Analysis (TCGA) and Beat AML to describe the association of *RARA* expression with survival and response to azacitidine in AML patients. RARA-high patients were predefined as patients whose *RARA* expression ranked in the top 30th percentile among non-acute promyelocytic leukemia (non-APL) AML patients in each dataset to be consistent with the RARA positivity rate observed in the AML patients screened in the SY-1425-201 study. In the TCGA dataset, analysis was limited to those patients ≥ 60 years of age, with intermediate or poor risk cytogenetics in order to analyze a population consistent with the characteristics of patients enrolled in the ND unfit AML cohort in this Phase 2 study (Supplemental Figure 2). In the Beat AML dataset, analysis was limited to patients ≥ 60 years of age without specifying cytogenetic risk, as cytogenetic risk was not an available variable in this dataset. Survival of the RARA-high and RARA-low populations was estimated using Kaplan-Meier methods and their survival was compared using a logrank test. Furthermore, patient treatment data from the Beat AML dataset were used to compare the complete remission (CR) rate

to azacitidine monotherapy in RARA-high and RARA-low patients using Fisher's exact test. *RARA* expression was determined using RNA-seq from patient samples as described above.

Monocytic Gene Expression Signature Development

Monocytic expression score (MES) was developed to distinguish M0/M1/M2 versus M4/M5 based on the RNA expression of 9 genes associated with monocytic differentiation in the TCGA dataset, with MES higher than 0.5 classified as monocytic (M4/M5) and lower than 0.5 as primitive (M0/M1/M2). It was further validated in the independent Beat AML dataset and achieved high specificity and sensitivity in classifying French, American, British classification (FAB) status.

To evaluate the relationships between *RARA* expression, monocytic AML features, and venetoclax resistance in our clinical study patients, a monocytic gene expression signature was developed to estimate the monocytic status of patient AML blasts collected at study entry. The TCGA LAML RNA-seq dataset was used to develop the signature through regularized regression of the expression of 9 well-established monocytic and primitive genes (CD14, CLEC7A, CD86, LYZ, MAFB, CD34, ITGAM, FCGR1A, and KIT) onto FAB across 130 non-APL AML patients with known FAB status. The logistic regression model used lasso regularization to distinguish FAB M4, 5 (monocytic) from FAB M0, 1, 2 (primitive). Ten-fold cross-validation was employed. The resulting model prediction was termed the MES, and samples with MES greater than 0.5 were predicted to be monocytic. The model achieved 88% sensitivity and 77% specificity cross-validated on TCGA data (Supplemental Figure 4). Its utility at discriminating monocytic from

primitive AML was further validated in an independent RNA-seq dataset from Beat AML, in which the MES yielded a 95% sensitivity and 72% specificity (Supplemental Figure 4). The MES was then applied to the RNA-seq datasets from ND unfit AML patients treated with tamibarotene plus azacitidine in the ongoing SY-1425-201 study.

The MES, *RARA* expression, and venetoclax resistance-associated features were compared using Spearman's rho correlation; the association of the MES with the RARA biomarker and with International Working Group (IWG) clinical responses in tamibarotene plus azacytidine-treated patients was evaluated using a two-tailed t-test. Area under the curve (AUC) of cell viability curves were used to evaluate *ex vivo* sensitivity to compounds, including venetoclax, in the Beat AML dataset.

Methods to Evaluate Gene Expression

RNA-seq Processing

RNA from blood samples were extracted using the blood-based biomarker test and were used (inputs ranged 25 ng to 100 ng) for library preparation with the Roche KAPA RNA HyperPrep kit with RiboErase (HMR) kit. Following library preparation, all generated libraries underwent quality assessment before being loaded onto a NovaSeq 6000 and sequenced with a total read length of 1×76 bp and to a target read budget of on average 50 million single reads per sample. Raw sequence data in FASTQ format was processed through quality control (QC) assessment steps

hosted on the DNAnexus cloud platform. Basic sequencing metrics such as GC content were calculated from unaligned reads using FastQC. RNA-seq data from TCGA and Beat AML patients with non-APL AML were downloaded and count level data were used. SY-1425-201 clinical study RNA-seq data were aligned to hg19 using RNA-seq by Expectation-Maximization (RSEM), and QC was performed with SeqQC. All data were processed with DESeq2 and transformed with DESeq2's variance stabilizing transformation. The datasets were then quantile normalized to the TCGA data for comparison.

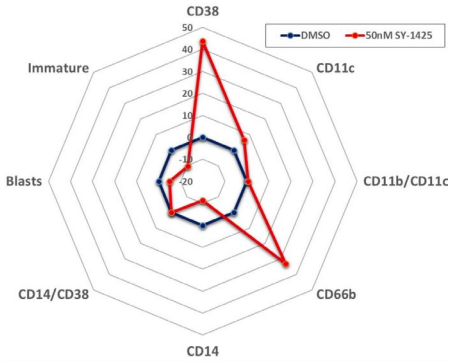
***Ex Vivo* Differentiation Supports RARA Biomarker Cutoff**

Patient blood samples were evaluated for differentiation responses of blasts to tamibarotene *ex vivo* (Jurcic et al, ASH 2017, Abstract 2633). The early induction of myeloid differentiation observed in RARA-positive patients demonstrated decreased immature cell population (e.g., CD33+ and LIN-) and increased cells with maturation markers (e.g., CD38) in response to tamibarotene, consistent with previously reported cell line and *ex vivo* differentiation studies (Supplemental Figure 1) (McKeown et al, Cancer Discov. 2017, doi: 10.1158/2159-8290; McKeown et al, EHA 2017, Abstract E884). The random forest machine learning model used to predict RARA biomarker status was able to achieve significant separation of scores by RARA biomarker status ($P < 5\times10^{-5}$, two-tailed t-test), demonstrating that a phenotype of differentiation following exposure to tamibarotene is strongly associated with RARA positivity at baseline (Supplemental Figure 1) (Jurcic et al, ASH 2017, Abstract 2633).

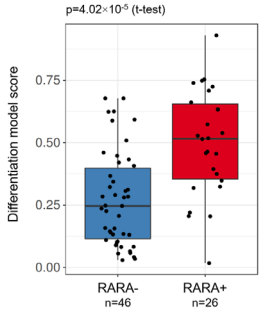
SUPPLEMENT – FIGURES

Supplemental Figure 1 Myeloid differentiation of blast cells in response to tamibarotene

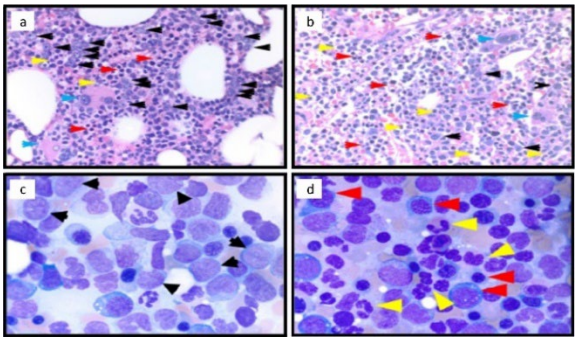
1A) *Ex vivo* immunophenotyping of an example clinical study patient sample (R/R AML) following tamibarotene treatment demonstrates differentiation

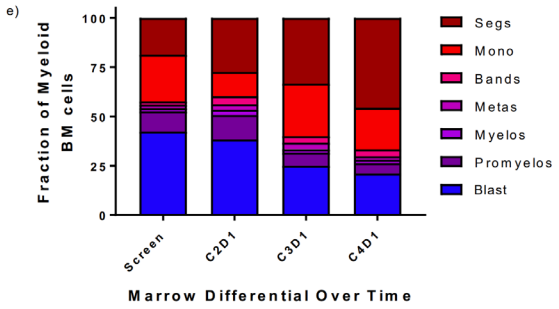


1B) *Ex vivo* myeloid differentiation in response to tamibarotene predicts RARA biomarker status



1C) Example of myeloid differentiation in a patient with R/R AML treated with tamibarotene monotherapy in the SY-1425-201 clinical study





BM, bone marrow; FAB, French, American, British classification; H&E, hematoxylin and eosin staining; R/R, relapse/refractory

Panel 1A: Radar plot showing the immunophenotyping of a biomarker positive patient sample collected at screening from the SY-1425-201 study and treated with 50 nM tamibarotene *ex vivo* for 72 hours (included in the poster for McKeown, EHA 2017, Abstract E884). The radar plot values represent percent change vs DMSO vehicle control. Blast and Immature Blast calculated as the percent change in the ratio of blast cell count to total live cell count. CD markers calculated as the percent change in marker count to total live blast count (McKeown et al, EHA 2017, Abstract E884).

Panel 1B: Box and whisker plot showing the difference in random forest differentiation predictions (y axis) for biomarker positive and negative (x axis) *ex vivo* patient samples. RARA+ predictions are higher ($P = 4.02\times10^{-5}$, t-test).

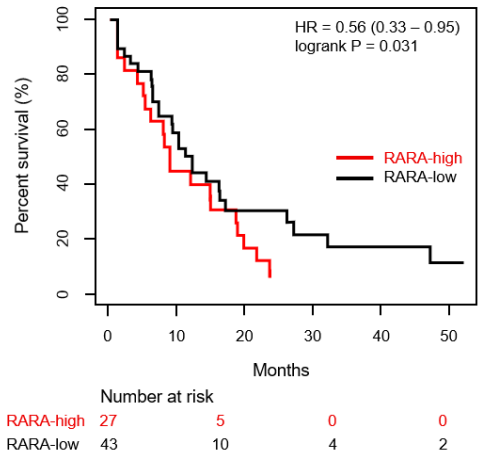
Panel 1C: 66-year-old male with R/R AML (FAB M5), intermediate risk cytogenetics (normal) and *IDH2* mutation. Myeloid differentiation with >25% marrow blast reduction noted at C3D1, continuing C4D1. Bone marrow biopsies (H&E staining) at 40x from screening (1C-a) and C3D1 (1C-b). Bone marrow aspirate (WG statin) at 100x from screening (1C-c) and C3D1 (1C-dy) (Jurcic et al, ASH 2017, Abstract 2633).

Panel 1C-a: Blasts (black arrows) occur in many small groups. Maturing myeloid cells (yellow arrows), erythroid precursors (red arrows), and megakaryocytes (blue arrows) are also present. **Panel 1C-b:** Fewer blasts (black arrows) scattered singly with higher number of maturing myeloid cells (yellow arrows), erythroid precursors (red arrows), and megakaryocytes (blue arrows). **Panel 1C-c:** Black arrowheads = blasts, myeloid and monocytic. **Panel 1C-d**: Yellow arrowheads = granulocytes. Red arrowheads = erythroid precursors.

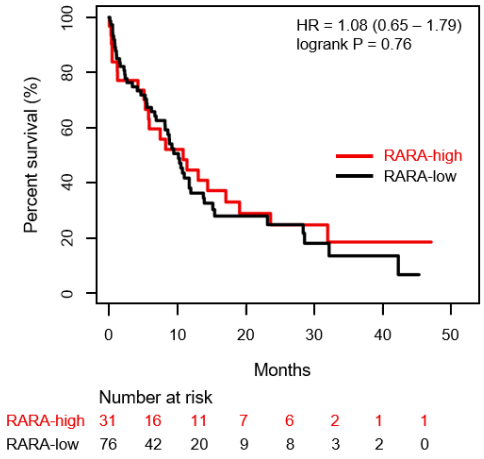
Panel 1C-e: Myeloid differentiation was seen at the time of marrow response assessments, with myeloblast percentage of myeloid cells decreasing from 25% to 12% and segmented neutrophils increasing from 12% to 28% between screening and C4D1.

Supplemental Figure 2 Prognostic Significance of RARA Biomarker in AML

2A) **TCGA: Patients ≥ 60 years of age with intermediate/poor risk cytogenetics**



2B) **Beat AML: Patients ≥ 60 years of age**



AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CR, complete remission; HR, hazard ratio; RNA-seq, RNA sequencing; TCGA, The Cancer Genome Atlas

2C) **Response to Azacitidine Monotherapy in Patient Samples from Beat AML Dataset**

Genotype	n	CR	Any Other Response	CR% (95% CI)	P-value*
RARA-high	17	5	12	29 (13 to 53)	P = 0.73
RARA-low	37	8	29	22 (11 to 37)	

AML: acute myeloid leukemia; CI: confidence interval; CR: complete remission; RARA: gene encoding retinoic acid receptor-a. Any Other: partial response or lack of response.

* Comparison of the response between RARA-positive and RARA-negative patients to azacitidine monotherapy; Fisher's exact test.

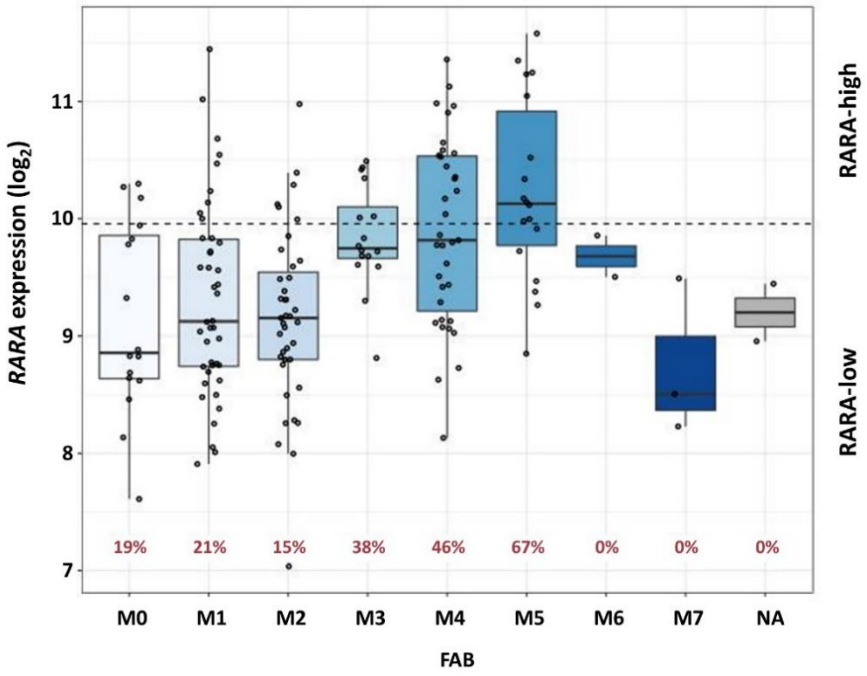
RNA-seq datasets were evaluated from TCGA and Beat AML databases to describe the associated of *RARA* expression with survival and response to azacitidine in AML patients. RARA-high patients were defined as patients whose *RARA* expression ranked in the top 30th percentile among non-APL AML patients in each dataset to be consistent with the RARA positivity rate observed in the AML patients screened in the SY-1425-201 study.

Panel 2A: *RARA* expression was not associated with the Beat AML patients' CR rate to azacitidine monotherapy. RNA-seq data were generated from a combination of the Beat AML patients' bone marrow and peripheral blood samples from non-APL AML patients. Patients with *RARA* expression in the top 30th percentile were considered RARA-high.

Panel 2B: There was statistically significant inferior survival in RARA-high compared to RARA-low patients from the TCGA database when restricting to ≥ 60 years of age and those with intermediate/poor risk cytogenetics.

Panel 2C: Survival was comparable among RARA-high and RARA-low patients in the Beat AML patients when restricting to ≥ 60 years of age.

Supplemental Figure 3 *RARA* expression is highest in monocytic AML (FAB M4, M5)

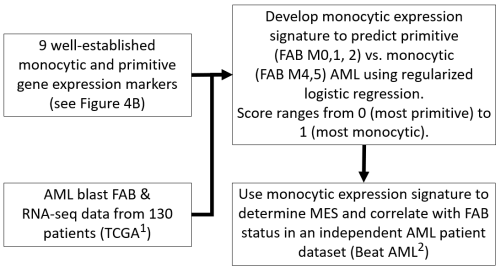


AML, acute myeloid leukemia; FAB, French, American, British classification; RNA-seq, RNA sequencing; TCGA, The Cancer Genome Atlas

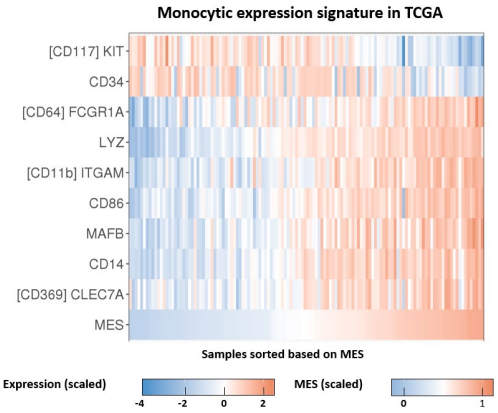
RARA expression is normalized against the expression of all genes using the RNA-seq data from TCGA AML patients (Cancer Genome Atlas Research Network et al, N Engl J Med. 2013; doi: 10.1056/NEJMoa1301689). The dashed horizontal line identifies the predefined upper 30th percentile of *RARA* expression (RARA-high) across all patients, consistent with the RARA positivity rate (30%) observed with the RARA biomarker test in AML patients enrolled in the SY-1425-201 clinical study.

Supplemental Figure 4 Development of the MES to assess monocytic status of AML patient samples

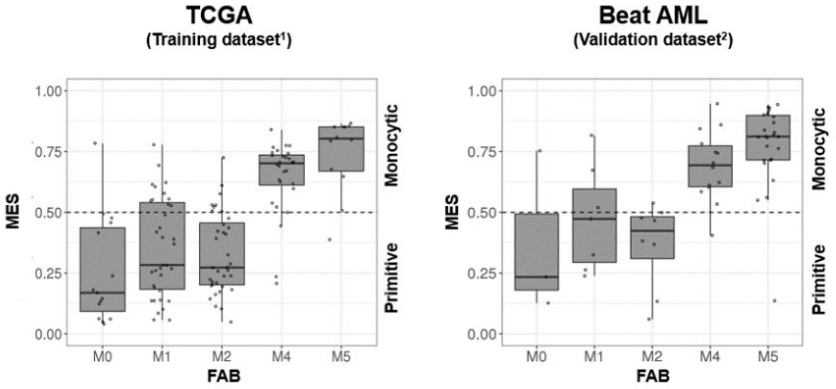
4A) Development of monocytic expression score (MES)



4B) Monocytic gene expression signature in TCGA



4C) High monocytic expression score (MES) correlates with monocytic FAB status



MES performance when using MES > 0.5 to classify samples as monocytic (FAB M4/M5)

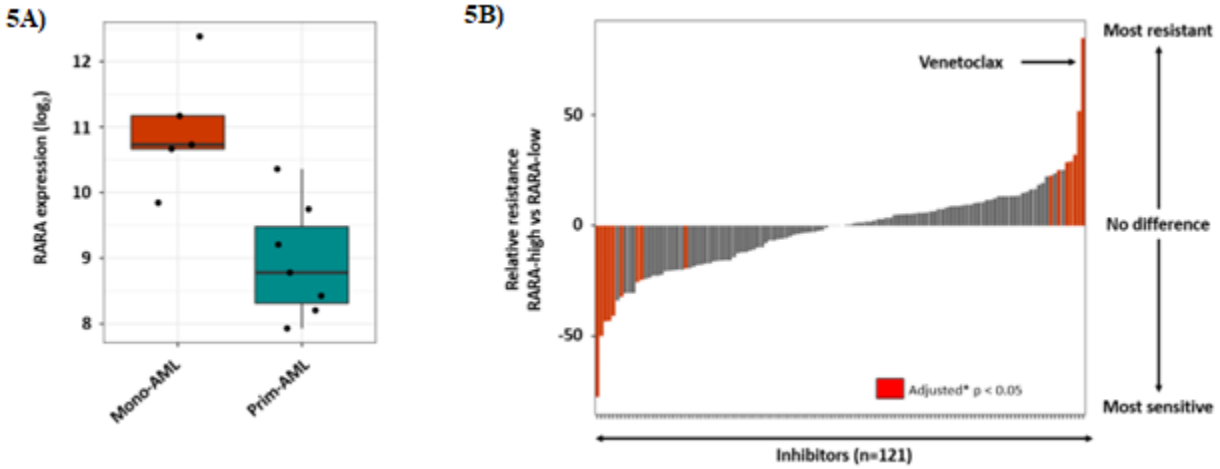
	TCGA (Training dataset)	Beat AML (Validation dataset)
Sensitivity	88%	95%
Specificity	77%	72%

AML: acute myeloid leukemia; FAB: French, American, British classification; MES: monocytic expression score; RNA-seq: RNA sequencing; TCGA: The Cancer Genome Atlas

1. Cancer Genome Atlas Research Network et al, N Engl J Med. 2013; doi: 10.1056/NEJMoa1301689
2. Tyner et al, Nature 2018; doi: 10.1038/s41586-018-0623-z

Panel 4A: The process to develop and validate the MES to analyze AML patient samples is shown.
Panel 4B: TCGA RNA-seq was used to develop the MES through expression analysis of 9 well-established monocytic and primitive genes across 130 AML patients with known FAB status. The 9 genes are shown as rows with TCGA patient samples as columns. The colors denote expression of each gene in each sample, row normalized (centered and variance standardized), except for the final MES row, which shows the MES as a color gradient from 0 (blue) to 1 (red).
Panel 4C: The MES used a logistic regression model with lasso regularization to distinguish FAB M4/5 (monocytic) from FAB M0/1/2 (primitive) and achieved 88% sensitivity and 77% specificity cross-validated on the TCGA data. The utility of the MES at identifying monocytic from non-monocytic AML was validated in an independent dataset from Beat AML, in which the MES had a 95% sensitivity and 72% specificity using a score > 0.5 to classify samples as monocytic.

Supplemental Figure 5 Association between high *RARA* expression and venetoclax resistance in cell-based models derived from AML patients



AML, acute myeloid leukemia; AUC, area under the curve; LSC, leukemic stem cell; Mono-AML, monocytic AML; non-APL, non-acute promyelocytic leukemia; Prim-AML, primitive AML

Panel 5A: *RARA* expression in monocytic- and primitive-AML patient LSC samples based on an analysis of data from LSCs resistant to venetoclax and azacitidine (Pei et al, Cancer Discov. 2020;doi: 10.1158/2159-8290.CD-19-0710).
Panel 5B: Non-APL AML primary cultures with high *RARA* expression are resistant to venetoclax *ex vivo*. Dose-response curves of 121 inhibitors were analyzed across all available primary cultures derived from AML patients (there were 90 such cultures available for venetoclax), with relative resistance defined as averaged AUC differences between RARA-high and RARA-low samples. High *RARA* expression is defined as upper 30th percentile of *RARA* expression (Tyner et al, Nature 2018; doi: 10.1038/s41586-018-0623-z).