Abstract #: 137323

Selection of RARA-positive Newly Diagnosed Unfit AML Patients with Elevated *RARA* Gene Expression Enriches for Features Associated with Primary Resistance to Venetoclax and Clinical Response to SY-1425, a Potent and Selective RARα Agonist, plus Azacitidine

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Disclosures

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Introduction

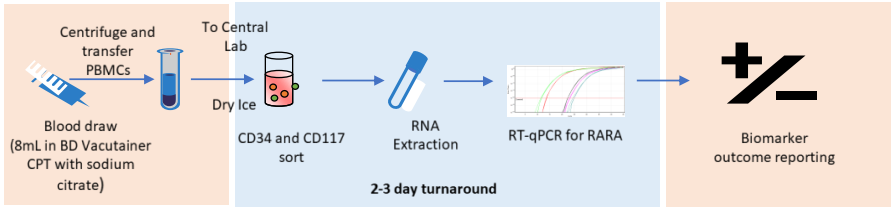
- SY-1425, an oral selective RARα agonist, is being developed in non-APL AML in combination with azacitidine (Aza) and demonstrates high rates of complete remission (CR) and deep molecular and cytogenetic CRs in RARA-positive newly diagnosed (ND) unfit AML[1]
- Mapping of transcriptionally active regulatory regions (super-enhancers, SE) in non-APL AML patient blasts identified a subset of patients with a SE associated with the *RARA* gene and elevated *RARA* expression, and a SE profile similar to mature monocytes[2]
- The BCL-2 inhibitor venetoclax (Ven) has emerged as a standard of care for treatment of patients with ND unfit AML in combination with hypomethylating agents (HMAs)[3]
  - Approximately one-third of patients do not respond to Ven plus HMAs including Aza [3,4], highlighting a continuing significant unmet need in ND unfit AML
  - Several reports have recently shown that primary Ven resistance is associated with monocytic features in AML[5-7] and that low-level monocytic clones present at diagnosis expand at relapse on Ven/Aza treatment[7]
- Here we report that RARA positivity significantly enriches for ND unfit AML patients with monocytic features associated with Ven resistance, highlighting the potential of SY-1425 in a patient population less likely to respond to Ven/Aza treatment, and for whom a high unmet need remains

[1]de Botton, Abstract 134600, ASH Annual Meeting 2020; [2]McKeown, Cancer Discovery 2017; [3]Dinardo, NEJM 2020; [4]Dinardo, Blood 2019; [5]Zhang, Blood 2018; [6]Kuusanmäki, Haematologica 2020; [7]Pei, Cancer Discovery 2020

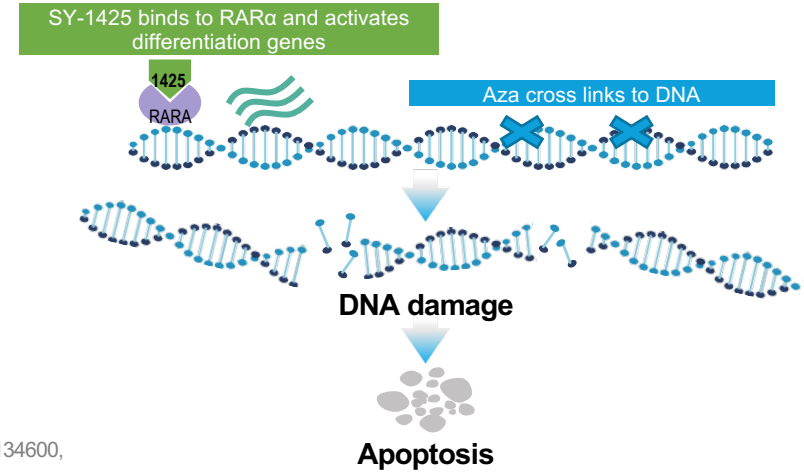
RARA-positive AML is a Novel Patient Subset with an Actionable Target for Treatment with SY-1425, an Oral, Selective RARα Agonist

- Subset of non-APL AML patients characterized by overexpression of the *RARA* gene
  - Novel blood-based biomarker test identifies patients for treatment with SY-1425, with typical 2 to 3-day turnaround time[1,2]
  - Approximately 30% of AML patients are RARA-positive[2]
- Preclinical synergy of SY-1425 with Aza supported development of the combination in RARA-positive myeloid malignancies[3]
- SY-1425 in combination with Aza demonstrates a high CR/CRi rate (61%), deep molecular and cytogenetic CRs (89%, 8 out of 9 CRs), and rapid onset of responses (1.2 months) in RARA-positive ND unfit AML[4]

Blood-based biomarker test for *RARA* gene expression



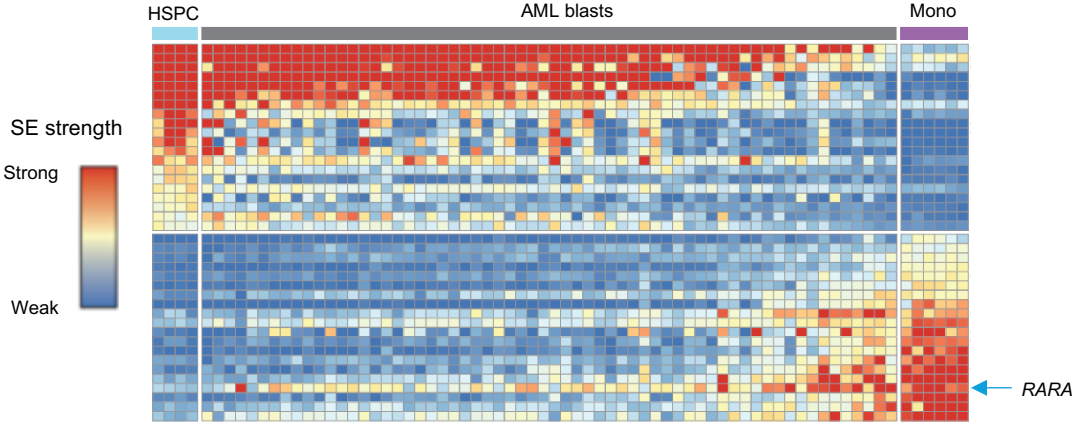
SY-1425 enhances apoptosis preclinically



[1]McKeown, Cancer Discovery 2017; [2]Vigil, ESH 2017; [3]McKeown, Haematologica 2018; [4]de Botton, Abstract 134600, ASH Annual Meeting 2020

RARA Super-enhancer and High *RARA* Expression are Associated with Monocytic Features in AML

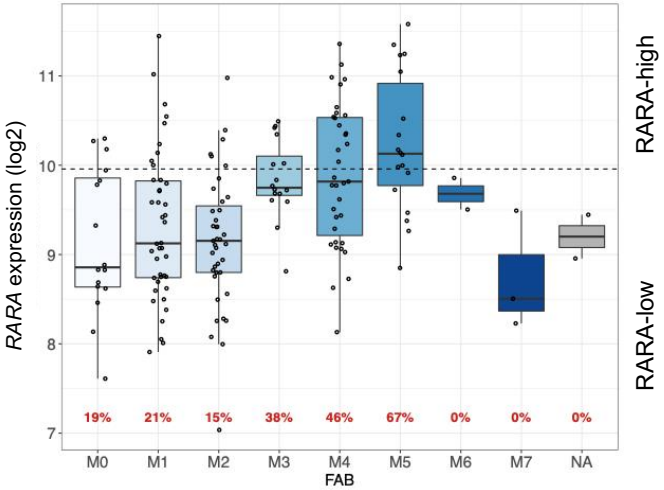
Super-enhancer mapping identified RARα as a novel target in an AML patient subset enriched for monocytic features



The primary AML blast samples are sorted left to right based on increasing similarity to the monocyte SE profile. Each column represents a unique sample. The 20 most HSPC-associated and 20 most monocyte-associated SEs are represented by each row, with the *RARA* SE as noted.
SE: super-enhancer; HSPC: hematopoietic stem & progenitor cell; Mono: monocyte

- A subset of AML patients have a super-enhancer (SE) associated with the *RARA* gene
  - SE profile is similar to mature monocytes
  - *RARA* expression is elevated in AML blasts with a high *RARA* SE score[1]

RARA gene expression is highest in monocytic AML (FAB M5)



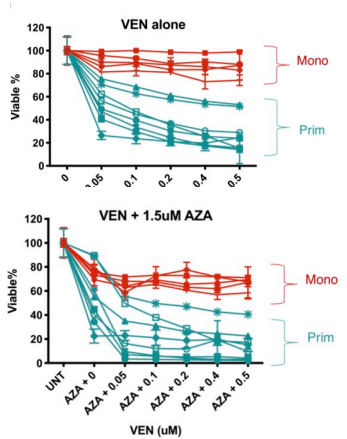
- *RARA* expression is normalized against the expression of all genes using the RNA-seq data from TCGA AML patients[2]
- Dashed horizontal line delineates the upper 30th percentile of *RARA* expression (RARA-high) across all patients, consistent with the RARA positivity rate (30%) observed with the RARA biomarker clinical trial assay in AML patients screened for enrollment in the SY-1425-201 clinical study

[1]McKeown, Cancer Discovery 2017; [2]Ley, NEJM 2013

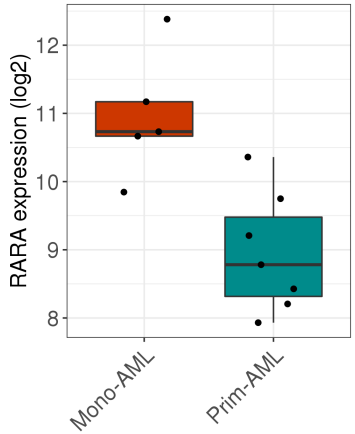
High *RARA* Expression is Associated with Ven Resistance in Cell-based Models Derived from AML Patients

High *RARA* expression observed in leukemic stem cells resistant to Ven ± Aza

Ven ± Aza dose-response curves in monocytic- and primitive-AML patient samples (Pei et al. 2020)

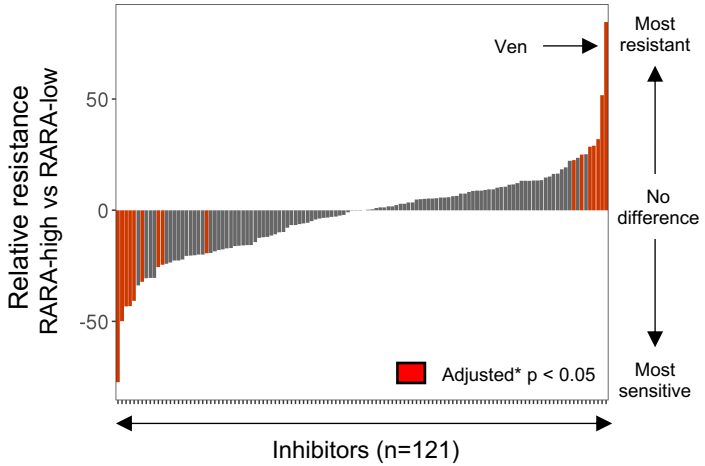


RARA expression in monocytic- and primitive-AML patient samples



- Leukemic stem cells were isolated and treated *ex vivo* with Ven ± Aza[1], and their *RARA* expression was normalized against the expression of all genes using the RNA-seq data (GEO GSE132511)

AML primary cultures with high *RARA* expression are resistant to Ven *ex vivo*



- Published dose-response data of 121 inhibitors were analyzed across blast cultures derived from AML patients[2], with relative resistance defined as averaged AUC differences between RARA-high and RARA-low samples
- RARA-high: upper 30th percentile of *RARA* expression across all patient samples

[1]Pei, Cancer Discovery 2020; [2]Tyner, Nature 2018

Monocytic Expression Score (MES) based on Gene Expression Markers is Highly Correlated with FAB Status in AML Blasts

Development of monocytic expression score (MES)

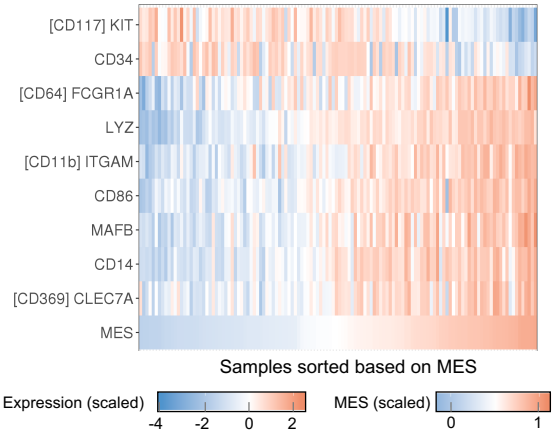
9 well-established monocytic and primitive gene expression markers (see figure below)

AML blast FAB & RNA-seq data from 130 patients (TCGA[1])

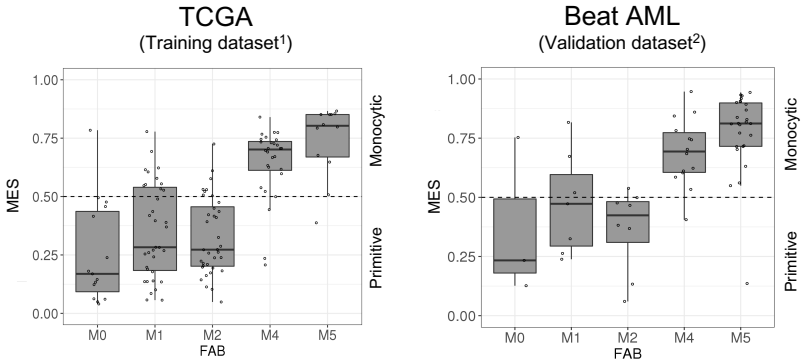
Develop monocytic expression signature to predict primitive (FAB M0,1, 2) vs. monocytic (FAB M4,5) AML using regularized logistic regression. Score ranges from 0 (most primitive) to 1 (most monocytic).

Use monocytic expression signature to determine MES and correlate with FAB status in an independent AML patient dataset (Beat AML[2])

Monocytic expression signature in TCGA



High MES correlates with monocytic FAB status



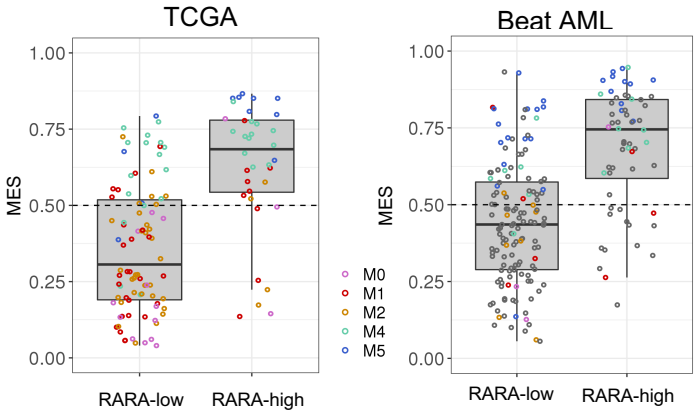
MES performance when using
MES > 0.5 to classify samples as monocytic (FAB M4/M5)

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

[1]Ley, NEJM 2013; [2]Tyner, Nature 2018

MES is Higher in AML Blasts with High *RARA* Expression, and Both *RARA* Expression and MES are Associated with Ven Resistance *ex vivo*

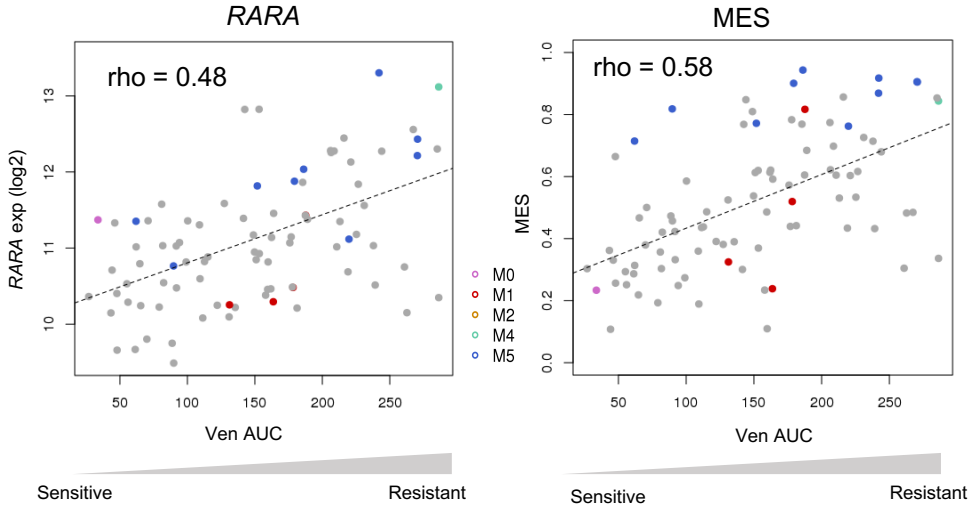
High *RARA* expression identifies an AML patient population enriched for high monocytic gene expression in TCGA and Beat AML databases



	% Monocytic (TCGA) p<1.2x10^{-7}	% Monocytic (Beat AML) p<1.6x10^{-7}
RARA-high	**81%** (29/36)	**77%** (46/60)
RARA-low	29% (26/90)	37% (51/139)

- *RARA* RNA-seq data from TCGA and Beat AML patients were normalized against the expression of all genes, with top 30% patients defined as RARA-high. P-values by Fisher's Exact test. Monocytic: MES > 0.5

RARA expression and MES are associated with Ven resistance *ex vivo*

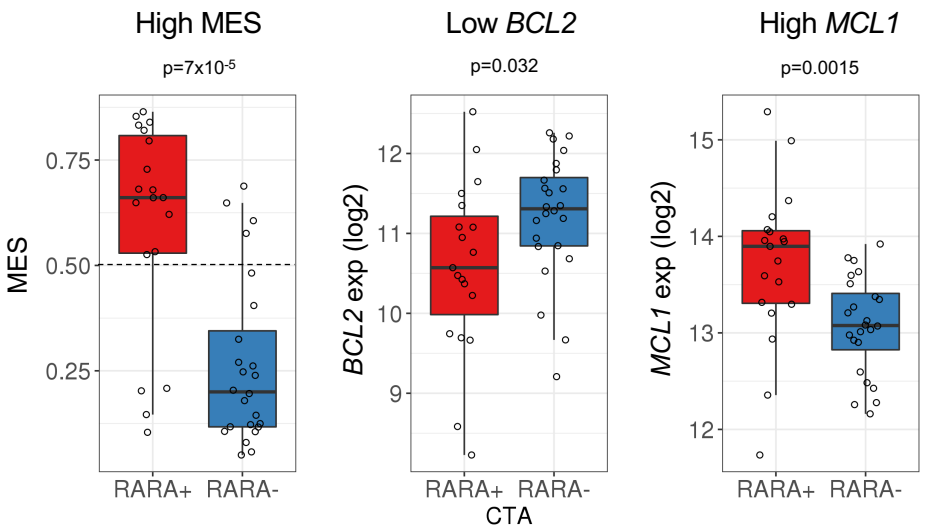


- Spearman correlation (rho) of normalized *RARA* expression (left) or MES (right) vs. Ven response across 90 AML primary cultures (Beat AML[1])

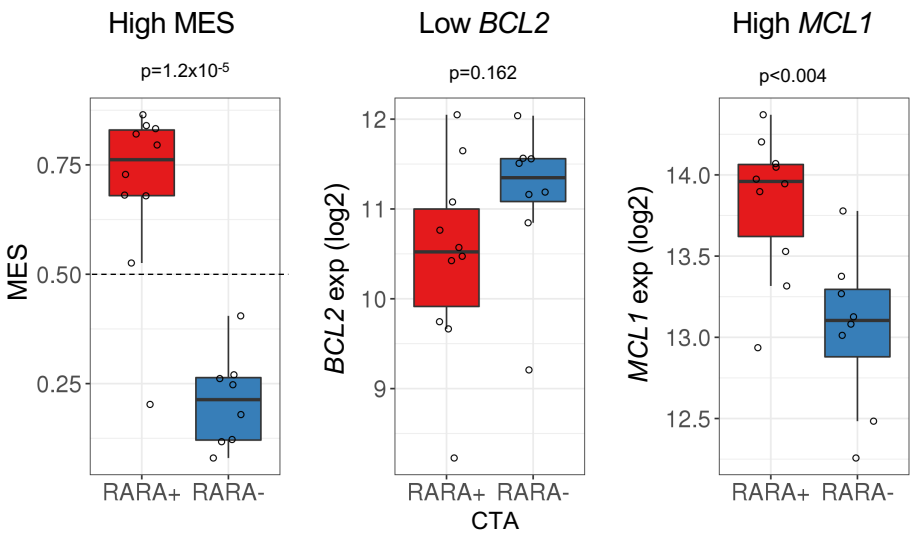
[1]Tyner, Nature 2018

RARA-positive ND Unfit AML Patients Including Those with Clinical Response to SY-1425 Plus Aza are Enriched for Features Associated with Ven Resistance

RARA-positive vs. RARA-negative ND unfit AML patients enrolled in SY-1425-201[1]



RARA-positive vs. RARA-negative ND unfit AML patients who achieved CR/CRi with SY-1425 plus Aza[1]



- 43 ND unfit AML patients enrolled in SY-1425-201 had RNA-seq data from blasts isolated with the RARA biomarker clinical trial assay (CTA)
  - 80% (15/19) of RARA-positive patients are classified as monocytic by MES (MES >0.5)
  - 17% (4/24) of RARA-negative patients are classified as monocytic by MES (MES >0.5)

[1]de Botton, Abstract 134600, ASH Annual Meeting 2020

Conclusions

- SY-1425, an oral selective RARα agonist, is being developed in combination with Aza in a subset of ND unfit AML patients selected by the RARA biomarker
  - Approximately 30% of AML patients are RARA-positive
  - SY-1425 plus Aza shows high CR rates, deep responses, and a rapid onset of response in RARA-positive ND unfit AML patients, with responses observed across cytogenetic risk groups and mutations[1]
- *RARA* is expressed at high levels in AML blasts with a monocytic phenotype (FAB M5) and is associated with Ven resistance in AML models and AML patients
- ~80% of RARA-positive ND unfit AML trial patients have monocytic phenotype associated with Ven resistance, which includes lower *BCL2* and higher *MCL1* expression
- RARA-positive ND unfit AML patients with features of Ven resistance respond to treatment with SY-1425 plus Aza with a high CR/CRi rate, suggesting an opportunity to address an emerging unmet need with SY-1425 combinations
- These data support the potential importance of identifying RARA-positive AML patients for combination treatment with SY-1425 in the ND unfit AML setting
- Further development of SY-1425 combinations is warranted in genomically defined RARA-positive AML patients, including those who may be resistant to Ven treatment

[1]de Botton, Abstract 134600, ASH Annual Meeting 2020