# RARA Pathway Activation Biomarkers in Study SY-1425-201 Define a New Subset of AML and MDS Patients and Correlate with Myeloid Differentiation Following Ex Vivo SY-1425 Treatment

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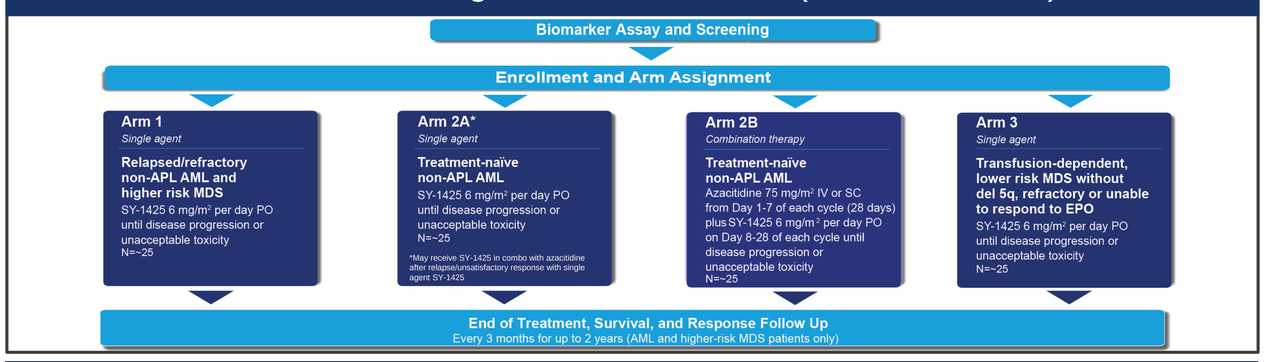
**Abstract 8882**

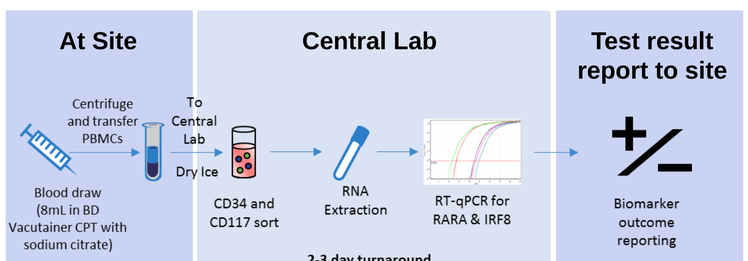
## Abstract

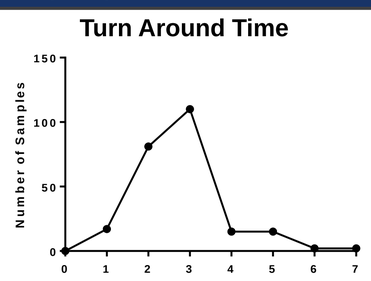
**Background:** We developed an epigenetic approach to profile the gene regulatory landscape of primary AML/MDS patient samples. A novel patient subset defined by RARA pathway activation was identified by super-enhancers (SEs) at the *RARA* and *IRF8* gene loci. These SEs were well correlated with mRNA expression of each gene, and predictive of preclinical response to SY-1425 (tamibarotene), a potent and selective RARα agonist. This discovery formed the basis of a biomarker selection strategy employed in an ongoing Phase 2 clinical study of SY-1425 in AML and MDS patients (NCT02807558).
**Objectives:** We used a novel biomarker test to evaluate the prevalence of RARA pathway activation in AML and MDS patients screened for enrollment in the Phase 2 study. Furthermore, we correlated biomarker status with *ex vivo* myeloid differentiation response to SY-1425 treatment, to support the predictive value of the biomarker.
**Methods:** A Clinical Trial Assay (CTA) was developed and received FDA Investigational Device Exemption approval for patient screening. RARA and IRF8 mRNA qPCR was performed on patient blasts enriched from Ficolled peripheral blood samples. The positivity cutoff was established using functional models characterized for SY-1425 response and retrospective analyses of patient expression databases. A set of 72 fresh patient samples were also analyzed for *ex vivo* differentiation by flow cytometry after exposure to SY-1425 at 50nM for 72 hours.
**Results:** Out of 152 patients screened (~70% AML, ~30% MDS), an overall biomarker positive rate of 37.2% was found. Both groups showed similar rates of each biomarker; RARA positive (19% AML, 19% MDS), IRF8 positive (5% AML, 7% MDS), and RARA/IRF8 double positive (8% AML, 19% MDS). This was consistent with an expected rate of 33-40% for the combined biomarkers based on large retrospective analyses of mRNA expression. In *ex vivo* studies, differentiation down multiple myeloid lineages was observed, including decreases in immature blasts and frequent CD38 induction. Two independent methods generating differentiation scores: an unbiased machine learning computational modeling approach, and an analysis of differentiation magnitude each identified a statistically significant separation of biomarker positive and negative patients, supporting the current cutoff.
**Conclusion**: A novel subset of AML and MDS patients dependent on RARA pathway activation was identified, providing rationale for a biomarker-directed clinical trial with SY-1425. The novel CTA prospectively identified 37% positivity for RARA, IRF8 or both. Correlation with *ex vivo* differentiation further supports the identification of a novel subset of patients who may benefit from SY-1425 treatment.

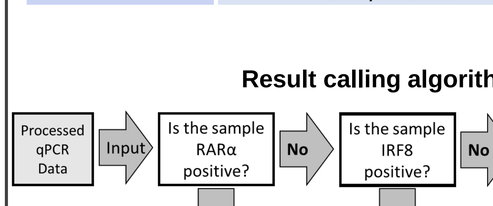
## Clinical trial design for SY-1425-201 (NCT02807558)




## Clinical trial assay for RARA pathway screening in peripheral blood

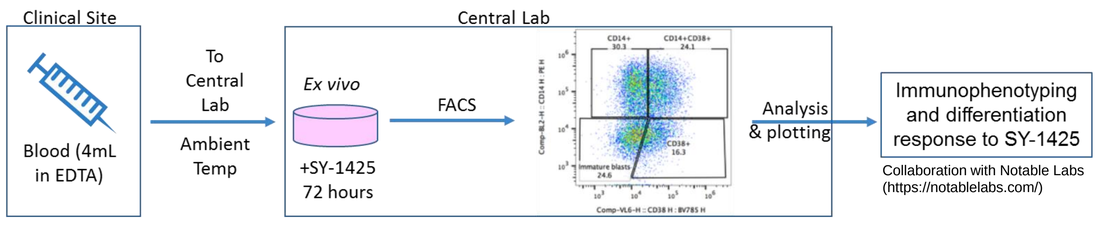



- Preclinically, super-enhancers were found to mark RARA and IRF8 genes in the RARA pathway and correlate with mRNA overexpression
- The IDE approved clinical trial assay utilizes a qPCR measurement for patient biomarker screening
- Measurements are performed on circulating blasts enriched by a CD34/CD117 based bead method
- qPCR quantifies RARA and IRF8 mRNA versus a control gene panel
- Rapid turn around time is achieved with an average of 2.8 days from sample reception at central lab
- Results are reported as RARA positive, IRF8 positive, or RARA & IRF8 negative based on a marker decision tree

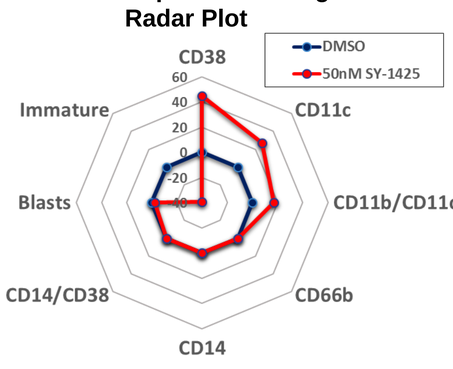
## *Ex vivo* analysis of patient samples support biomarker cut-off in current trial




Collaboration with Notable Labs (https://notablelabs.com/)

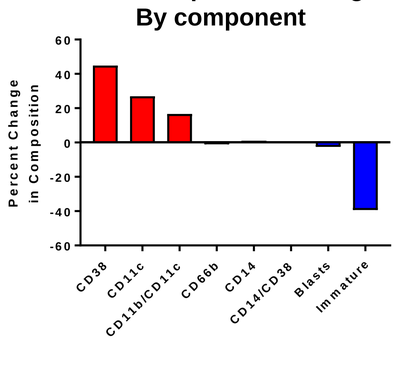
## Example of output from RARA+ patient

**Percent composition change – Radar Plot**




| Immunophenotyping | |
|---|---|
| Marker | Cell type |
| CD38 | Maturation |
| CD11c | Dendritic |
| CD11b/CD11c | Macrophage-like |
| CD66b | Granulocytic |
| CD14 | Monocytic |
| CD14/CD38 | Mature monocytic |
| Blasts | CD33+, CD34/CD117 |
| Immature | CD33+, Lin- |

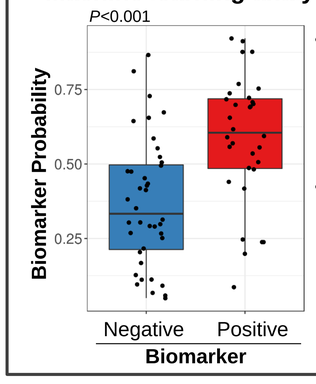
**Percent composition change – By component**




Blood samples from patients screened for entry into Study SY-1425-201 were analyzed for myeloid differentiation by flow cytometry following *ex vivo* exposure to 50 nM SY-1425 for 72 hours or DMSO control with analysis from one RARA+ patient depicted above. Analysis using multichannel flow cytometry assessed multiple metrics of differentiation based activity. Percent change in composition for radar (left) and bar plot (right) calculates the increase (positive) or decrease (negative) in the percent relative to DMSO control. Example shown here demonstrates evidence of reduced immature blast cells, induction of CD38, and multi-lineage maturation with increases in CD11c (dendritic) and CD11c/CD11b dual (macrophage-like) positive cells.

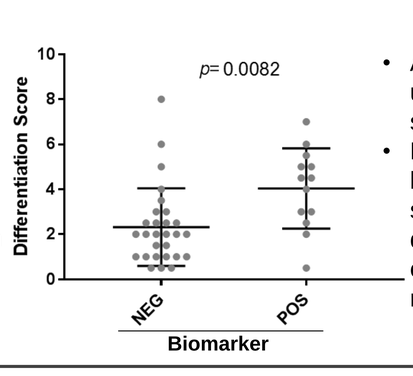
## Biomarker status associated with myeloid differentiation response

**Machine learning analysis**




- An unbiased machine learning approach was employed using a Random-Forest analysis
- Positive and negative biomarker samples significantly partition on the bases of flow cytometry marker changes

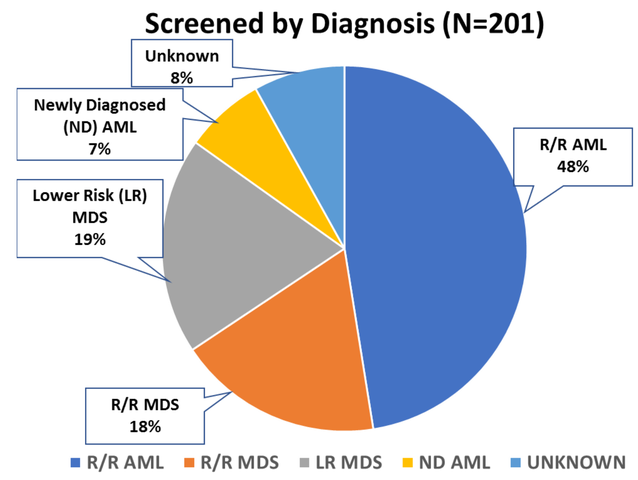
**Differentiation scoring algorithm**

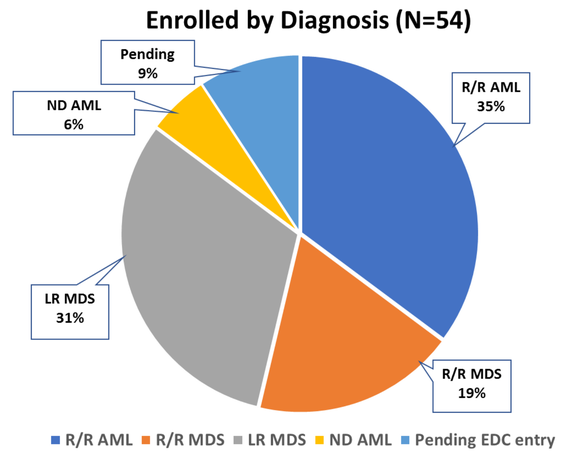


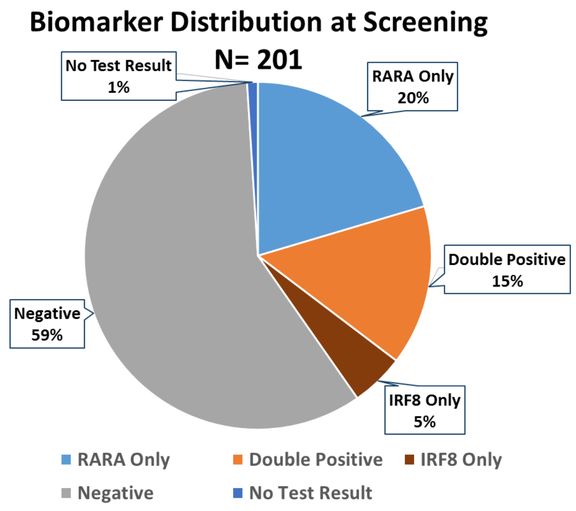

- A second approach used an algorithm for scoring differentiation
- POS and NEG biomarker patients showed significant differences in degree of differentiation response to SY-1425

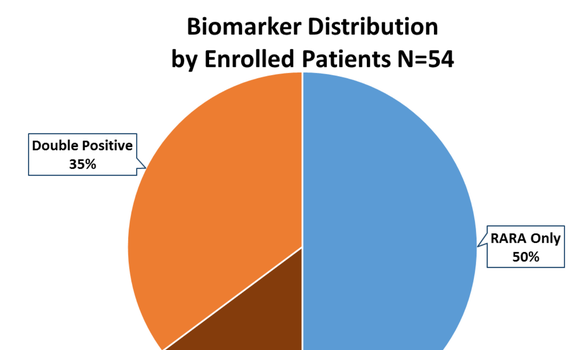
## Biomarker distribution and patient demographics

| Patient Demographics (N=201) | |
|---|---|
| Category | As of 8/31/2017 |
| **Age (y)** | |
| Mean (SD) | 72 (12) |
| Median | 74 (19, 94) |
| data *from 188 subjects* | |
| **Gender (%)** | |
| Male | 132 (66) |
| Female | 68 (34) |
| Not reported | 1 (<1) |
| **Diagnosis (%)** | |
| Relapsed/refractory AML | 94 (48) |
| Relapsed/refractory HR MDS | 37 (18) |
| Transfusion Dependent LR MDS | 38 (19) |
| Newly Diagnosed non-APL AML | 15 (7) |
| Not Reported | 17 (8) |
| **Biomarker (%)** | |
| RARA | 41 (20) |
| IRF8 | 10 (5) |
| Double | 30 (15) |
| Negative | 118 (59) |
| No Test Result | 2 (1) |



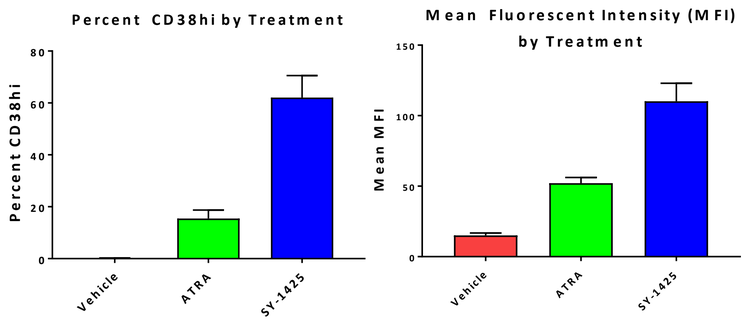

| Biomarker by Diagnosis [N(%)] | | | | |
|---|---|---|---|---|
| Category | RARA only | IRF8 only | Double+ | Negative |
| R/R AML* | 11 (12) | 4 (4) | 10 (11) | 67 (71) |
| HR MDS | 4 (11) | 2 (5) | 7 (19) | 24 (65) |
| ND AML | 4 (27) | 1 (7) | 2 (13) | 8 (53) |
| LR MDS | 16 (42) | 1 (3) | 7 (18) | 14 (37) |

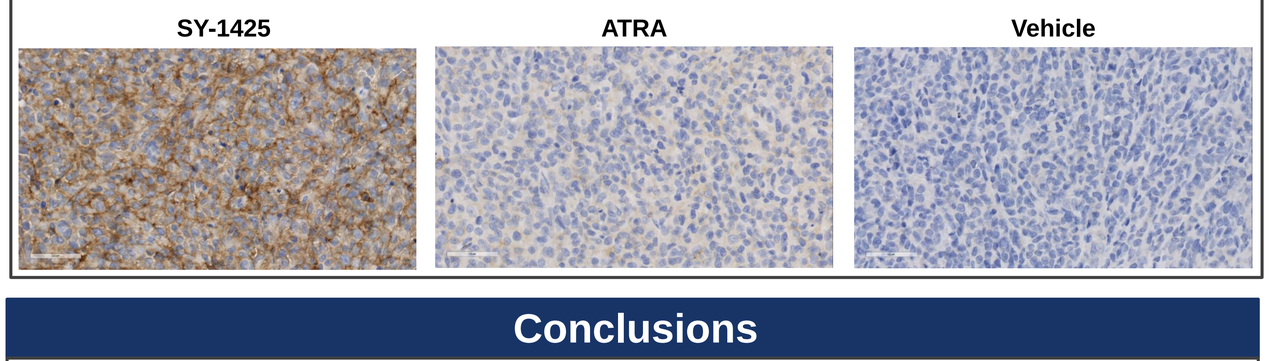
*2 R/R AML had no test result; 17 patients had not reported diagnosis at time of data cutoff

- Overall, 40% of screened patients were biomarker positive (201 evaluable patients were screened through August 31, 2017), including approximately one-third of R/R AML and HR MDS patients.
- The prevalence of biomarker positive results was consistent with expectations from preclinical models and analysis of historical databases.
- In biomarker positive patients, a similar distribution of RARA only, IRF8 only, or double positive, respectively, was found across AML and MDS.

## SY-1425 robustly induces CD38 vs ATRA in preclinical *in vivo* models of biomarker positive AML

- RARA-high MV411 AML cell line xenograft model treated with SY-1425, ATRA or vehicle at dosing matched to human daily clinical exposures.
- Following three weeks of continuous daily treatment for either drug, IHC stained tumor (below) demonstrated robust CD38 expression in SY-1425 treated mice. CD38 expression was minimal or negative in ATRA and vehicle treated mice, respectively.
- Flow cytometry of tumor cells demonstrated a significant increase of CD38hi (gated above baseline expression range) and MFI in SY-1425 treated tumor cells vs. ATRA or vehicle




## Conclusions

- Syros has developed a biomarker assay for AML and MDS patient screening based on preclinical super-enhancer analyses, to evaluate RARA pathway activation in peripheral blood myeloid progenitor cells.
- The biomarker test is a qPCR based assay that evaluates RARA and/or IRF8 gene expression with a rapid turnaround time of less than 3 days on average to report results.
- Evaluation of 201 patients screened for entry into Study SY-1425-201 (evaluable as of 8/31/2017) demonstrated an overall positive biomarker test result in 40% of patients with AML and MDS, including approximately one-third of R/R AML and HR MDS, consistent with expectations from preclinical studies and analyses of historical databases.
- Biomarker test results (positive vs. negative) were significantly associated with differentiation of patient blood samples following *ex vivo* treatment with SY-1425, supporting the clinical utility of the biomarker test for patient selection.
- CD38, a marker of myeloid maturation and target of anti-CD38 directed therapies, is strongly induced following SY-1425 treatment in patient samples *ex vivo*, and in mouse xenografts *in vivo* compared to ATRA or vehicle controls.
- Study SY-1425-201 is ongoing, and is exploring SY-1425 as a monotherapy and in combination with azacitidine across multiple patient populations. Based on the robust induction of CD38, a combination with an anti-CD38 therapeutic antibody is planned.

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