Abstract E884

SY-1425, A POTENT AND SELECTIVE RARA AGONIST, REPROGRAMS AML CELLS FOR DIFFERENTIATION ALONG DISTINCT LINEAGES, UNCOVERING PD MARKERS FOR CLINICAL STUDIES

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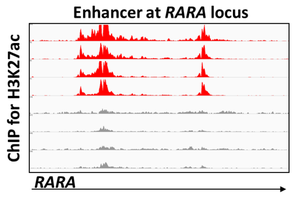
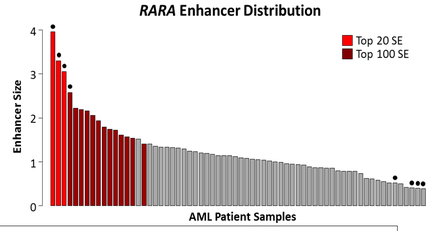
Abstract

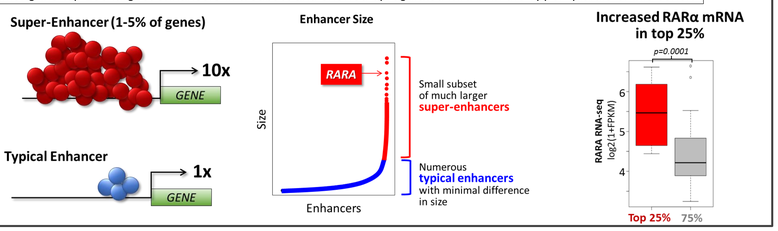
Background: SY-1425 (tamibarotene) is a potent and selective agonist of the retinoic acid receptor alpha (RARα) transcription factor (TF), currently in a biomarker directed Ph2 clinical study in AML and MDS patients (NCT02807558). A subset of AML and MDS has been found to have RARα pathway activation characterized by a large enhancer at the *RARA* locus (RARA-high) and/or upregulation of IRF8, a TF associated with RARα signaling, forming the basis of SY-1425 sensitive tumor identification.
Aims: We sought to understand how SY-1425 agonism of RARα acts to promote maturation and halt proliferation of AML blasts locked into an immature cell state by the cancer circuitry. This characterization could further inform clinical pharmacodynamics markers.
Methods: We analyzed the epigenomic and transcriptional landscape of 66 non-APL AML patients and normal primary myeloid cells by RNA-seq and ChIP-seq for the enhancer mark H3K27ac. AML cell lines were profiled by RNA-seq, ChIP-seq for H3K27ac and RARα, and ATAC-seq with or without SY-1425 treatment. Cell surface marker changes were assessed by flow cytometry.
Results: A subgroup of the patient samples was defined by an SE driving *RARA*, which co-occurred with SEs driving *FOS* and *JUNB,* or *IRF8*. FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crosstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models.
We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocytic, macrophage, dendritic, and granulocytic cell types. While APL has well characterized and specific granulocytic differentiation, we found that RARA/IRF8-high AML could follow multiple differentiation paths depending on the initial state of the AML model, necessitating different marker panels to capture full cell typing. Functional validation confirmed surface marker changes consistent with the observed epigenomic alterations including CD11b, CD11c, CD66b, and CD38 upregulation.
We integrated epigenomic data, DNA accessibility, and SY-1425 response to understand RARα agonist perturbation to cell circuitry. Enhancer elements directly bound by RARα were associated with greater response to SY-1425 as were enhancers bound by other TFs involved in myeloid differentiation. The accessibility of RAR elements and IRF motifs were increased and their associated TFs were upregulated. The target genes of known immature/proliferative state drivers, such as RUNX1 and CEBP, were downregulated. Importantly, the FOS/JUN circuit, identified as a component of the oncogenic RARα circuit in patient samples, was found to be suppressed.
Conclusion: As in normal myelopoiesis, RARα and associated cell state TFs play a critical role in the differentiation of AML. SY-1425 perturbation of this circuitry leads to differentiation toward multiple potential lineage paths depending on the initial state of the cancer. These pharmacodynamic changes can be assessed clinically and combined with common AML/MDS assays, such as blood and marrow aspirate smears or white blood cell differentials, to support the differentiation mechanism of action and offering the potential for early biologically relevant data to inform current and future clinical studies.

RARA pathway activation defines a subset of AML

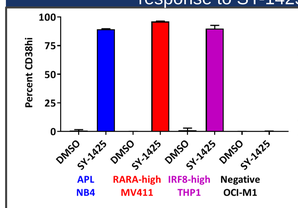




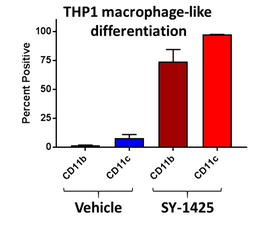
RARA has a super-enhancer in a subset of AML patients. In a cohort of 66 AML patients, the *RARA* gene was among the top 100 largest enhancers in a subset and showed upregulated mRNA in the upper quartile of this cohort



Biomarker-high cell lines demonstrate differentiative response to SY-1425

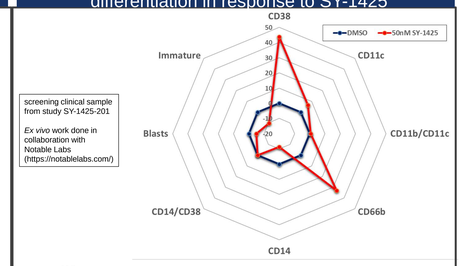


Flow cytometry showing induction of the CD38 maturation marker response to 50nM SY-1425 (24 h) in APL, RARA-high, and IRF8-high cell lines but not in a RARA-low, IRF-8 low cell line (OCI-M1).



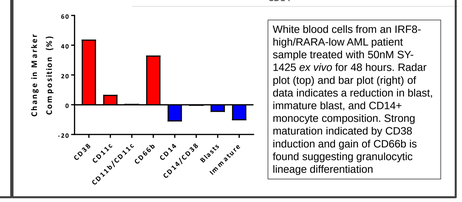
THP1s show differentiation toward a macrophage phenotype in response to 24hr 50nM SY-1425 as measured by CD11b and CD11c expression.

Ex vivo patient samples demonstrate evidence of differentiation in response to SY-1425



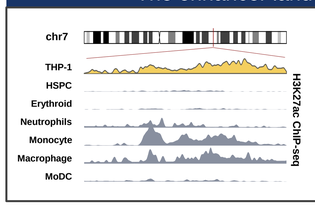
screening clinical sample from study SY-1425-201

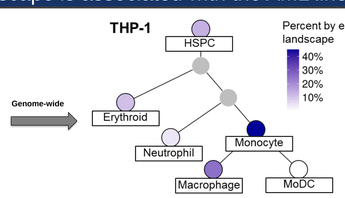
Ex vivo work done in collaboration with Notable Labs (https://notablelabs.com/)



White blood cells from an IRF8-high/RARA-low AML patient sample treated with 50nM SY-1425 *ex vivo* for 48 hours. Radar plot (top) and bar plot (right) of data indicates a reduction in blast, immature blast, and CD14+ monocyte composition. Strong maturation indicated by CD38 induction and gain of CD66b is found suggesting granulocytic lineage differentiation

The enhancer landscape is associated with the AML lineage state

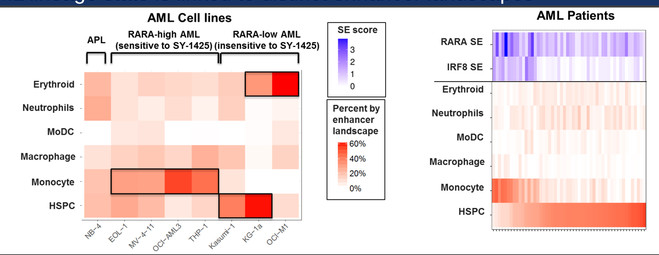




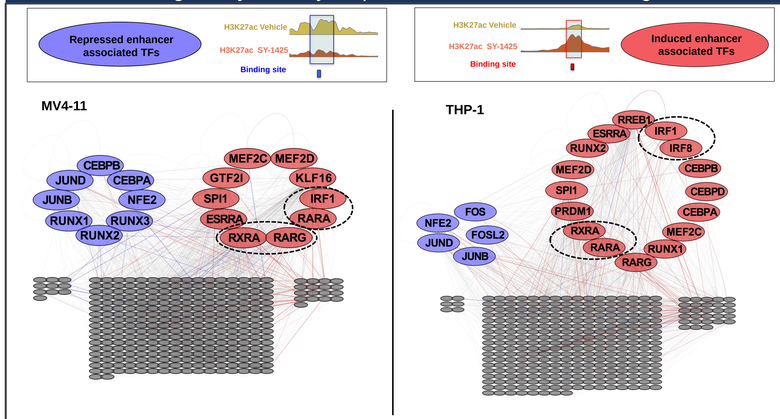
(Left) H3K27ac ChIP-seq tracks, 95kb upstream of the *CUX1* locus, in primary human blood samples and THP-1, an AML cell line.
(Right) Differentiation state of THP-1 as determined by the enhancer landscape.

AML lineage state is linked to distinct enhancer landscapes

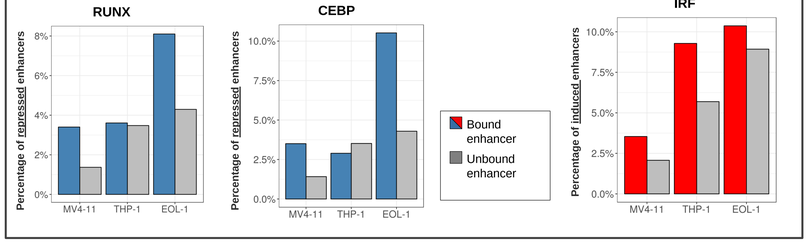
(Left) The differentiation state of AML cell lines by the enhancer landscape according to primary human blood samples.
(Right) The differentiation state of AML patient samples by their enhancer landscape. The SE score of RARA and IRF8 are also indicated.



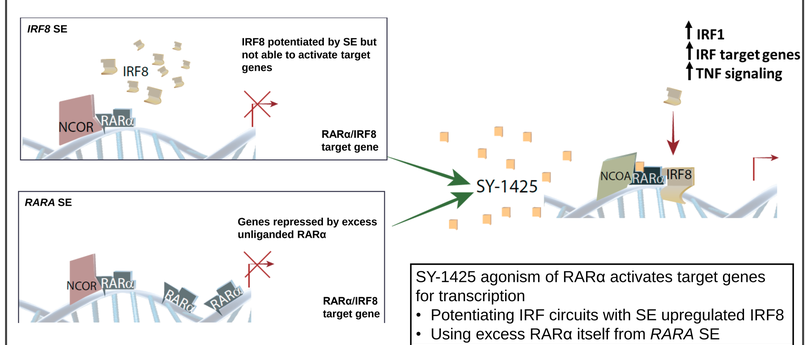
Gene regulatory circuitry response to SY-1425 in RARA-high AML



(Above) Differential gene regulatory circuitry in AML cell lines. Blue circles indicate TFs associated with repressed enhancers. Red circles indicate TFs associated with induced enhancers. Grey circles are other TFs. Lines between circles indicate that the TF is predicted to regulate the gene. A red line indicates regulation with an enrichment in induced regions, and a blue line indicates regulation with an enrichment in repressed regions.
(Below) Percentage of H3K27ac enhancers that are induced (right, with red bars) or repressed (left, with blue bars) according to whether they are predicted to be bound by the indicated binding site, in RARA-high AML cell lines.

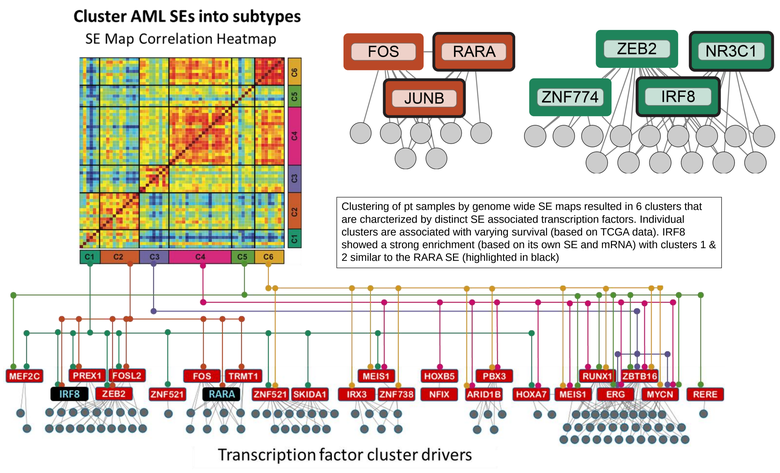


Model of RARA pathway activation and SY-1425 response



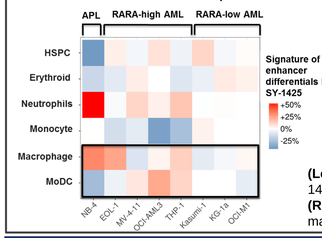
SY-1425 agonism of RARα activates target genes for transcription
- Potentiating IRF circuits with SE upregulated IRF8
- Using excess RARα itself from *RARA* SE

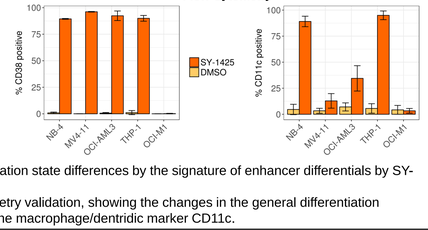
RARA pathway activated AML is defined by distinct nuclear circuitry



Clustering of pt samples by genome wide SE maps resulted in 6 clusters that are charcterized by distinct SE associated transcription factors. Individual clusters are associated with varying survival (based on TCGA data). IRF8 showed a strong enrichment (based on its own SE and mRNA) with clusters 1 & 2 similar to the RARA SE (highlighted in black)

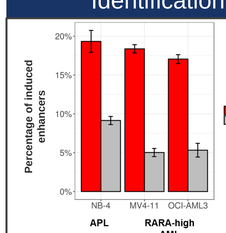
Shift in enhancer landscape by SY-1425 indicates AML cell differentiation





(Left) The differentiation state differences by the signature of enhancer differentials by SY-1425 treatment.
(Right) Flow cytometry validation, showing the changes in the general differentiation marker CD38 and the macrophage/dentridic marker CD11c.

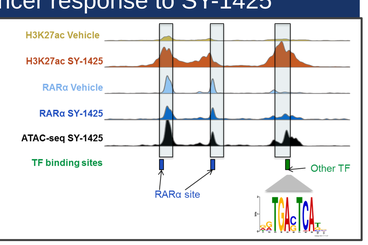
Identification of TFs involved in the enhancer response to SY-1425



(Left) The percent of H3K27ac enhancers induced upon SY-1425 treatment by whether they are bound by RARα.

RARα bound enhancers
Unbound enhancers

(Right) ChIP-seq track at the *DHRS* locus in MV4-11 cells showing an induced enhancer, RARα binding locations, and ATAC-seq regions of open chromatin.



Conclusions

- SY-1425 is a potent and selective RARα agonist with favorable PK properties
  - approved in Japan for the treatment of R/R APL, which is characterized by fusions between *RARA* and other transcription factor genes
- SY-1425 induces differentiation and anti-proliferative effects in non-APL AML preclinical models that are highly dependent on RARA pathway activation
  - RARα and IRF8 have a significant role in the gene regulatory circuitry of SY-1425 response.
  - Differentiation response to SY-1425 is mediatedby cell state changes in the TF usage, chromatin, and expression
  - Evidence of differentiation found in cell lines, PDX models, and *ex vivo* patient samples
- A biomarker-directed phase 2 trial (NCT02807558) of SY-1425 is currently ongoing in genomically defined subsets of AML and MDS patients.

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