Poster 06

SY-1425 (tamibarotene), a potent and selective RARα agonist, induces changes in the transcriptional regulatory circuit of AML cells leading to differentiation

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Abstract

SY-1425 (tamibarotene) is a potent and selective agonist of the transcription factor (TF) retinoic acid receptor alpha (RARα) that is currently being evaluated in a biomarker directed Ph2 clinical study in AML and MDS. A subset of AML and MDS patients, referred to as RARA-high, has previously been found to have a large enhancer (super-enhancer) at the RARA locus or upregulation of IRF8, a RARα associated TF. Here, we profile the non-coding genome and transcriptional landscape in AML cells to define the circuitry of RARA-high AML characterized by RARα pathway activation. In addition, AML cell lines were profiled with and without SY-1425 treatment to query the perturbations of key regulatory connections by SY-1425.

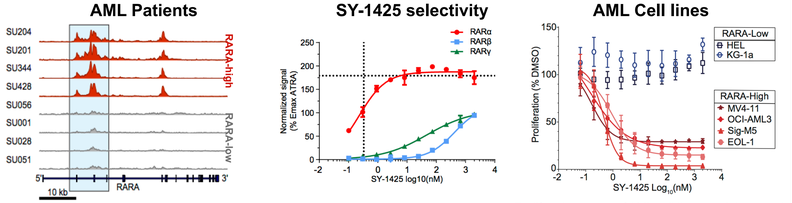
To better understand the regulatory circuitry of the RARα pathway in AML, we profiled RARA-high and RARA-low AML cell lines with a combination of ATAC-seq, H3K27ac ChIP-seq, and transcriptomic profiling. Using the enhancer landscape of these cell lines compared to a set of healthy human immune cells comprising hematopoietic stem and progenitor cells (HSPCs) as well as multiple myeloid lineages, we performed a non-negative least squares (NNLS) regression to determine the hematopoietic lineage components of these AML cell lines. We found that RARA-high cell lines sensitive to SY-1425 (THP-1, OCI-AML3, MV4-11, and EOL-1) tend to be most similar to monocytes, while RARA-low cell lines insensitive to SY-1425 (KG-1a, Kasumi-1, and OCI-M1) are more HSPC or erythroid-like.

We then studied the effect of SY-1425 on the differentiation state of these AML cell lines by additionally profiling them with and without treatment. We find that the enhancer landscape of RARA-high AML cell lines was changed by SY-1425 toward one resembling more fully differentiated myeloid cells of different subtypes, including dendritic cells, granulocytic cells, and macrophages. This was in contrast to the well-characterized granulocytic differentiation of APL caused by retinoids. Functional validation by flow cytometry confirmed surface marker changes consistent with the observed epigenomic alterations. Comparable changes were not found in RARA-low AML cell lines. This effect supports the hypothesis that SY-1425 treatment relieves a RARα mediated differentiation block of RARA-high AML.

To understand the individual regulatory connections being modulated in response to SY-1425, we investigated the binding locations of TFs in the RARA-high cell lines with and without SY-1425 treatment. As expected, enhancers and genes bound by RARα were associated with upregulation of transcription in response to SY-1425. TF binding sites for IRF1 and IRF8 were also identified at induced enhancers. Conversely, known factors important for maintaining an immature/proliferative state were found associated with enhancers most deactivated by SY-1425 (as measured by H3K27ac loss). These included RUNX1, CEBP, and members of the FOS/JUN circuit (which were noted previously as a component of the oncogenic RARα circuit in patient samples). Taken together, these results support that the mechanism of action for SY-1425 is through perturbation of myeloid regulatory relationships in RARA-high AML blasts, leading to a more differentiated phenotype.

Based on the understanding of AML and RARα driven tumor circuitry, we have generated insight into morphologic, lineage marker, and target-gene based measures to be used in conjunction with clinical studies. The ongoing Ph2 study of SY-1425 in AML and MDS (NCT02807558) will explore measurement of these features in patients.

Sensitivity to SY-1425 is associated with a super enhancer (SE) at the *RARA* locus in AML

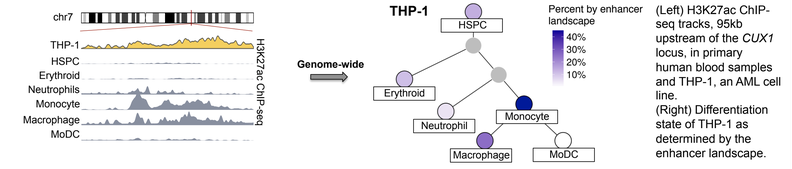


H3K27ac ChIP-seq tracks in AML patient samples, showing that an SE is present at the *RARA* locus in a subset of patients.

Selectivity of SY-1425 for RARs in a FRET assay.

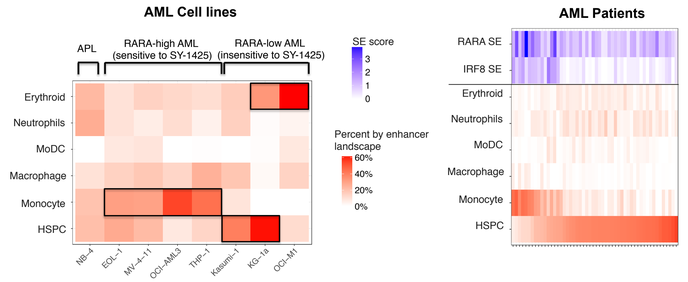
Proliferation assay of AML cell lines models treated with SY-1425. Those with RARA pathway activation (RARA-high) are sensitive to SY-1425, whereas those without (RARA-low) are insensitive.

The H3K27ac enhancer landscape is associated with the baseline differentiation state



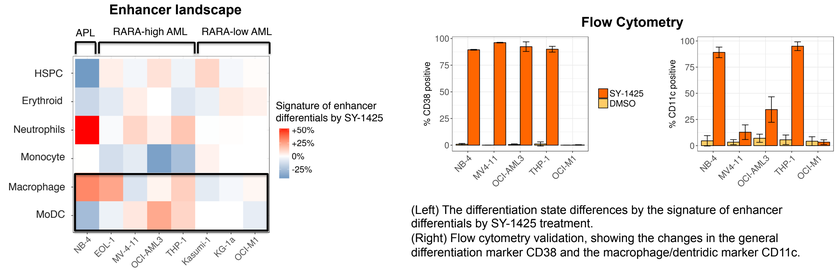
(Left) H3K27ac ChIP-seq tracks, 95kb upstream of the *CUX1* locus, in primary human blood samples and THP-1, an AML cell line.
(Right) Differentiation state of THP-1 as determined by the enhancer landscape.

Baseline differentiation state of AML by enhancer landscape in patients and cell lines



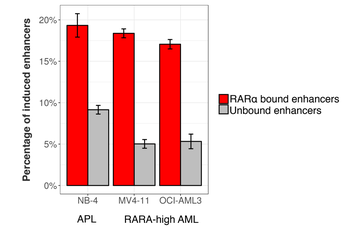
(Left) The differentiation state of AML cell lines by the enhancer landscape according to primary human blood samples.
(Right) The differentiation state of AML patient samples by their enhancer landscape. The SE score of RARA and IRF8 are also indicated.

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

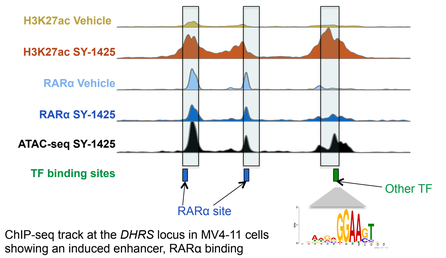


(Left) The differentiation state differences by the signature of enhancer differentials by SY-1425 treatment.
(Right) Flow cytometry validation, showing the changes in the general differentiation marker CD38 and the macrophage/dentridic marker CD11c.

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

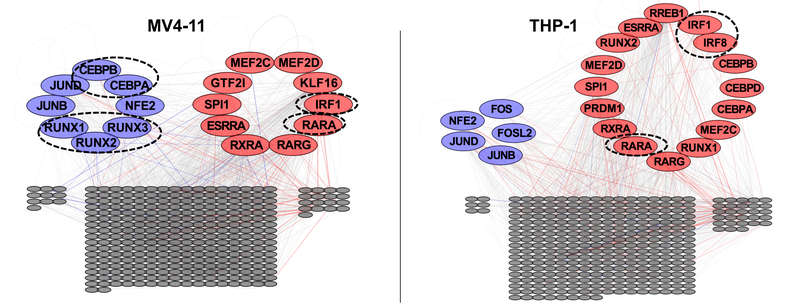


The percent of H3K27ac enhancers induced upon SY-1425 treatment by whether they are bound by RARα.

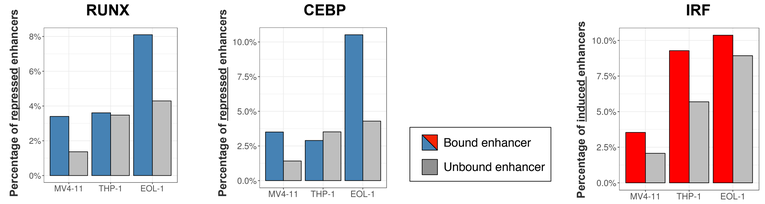


ChIP-seq track at the *DHRS* locus in MV4-11 cells showing an induced enhancer, RARα binding locations, and ATAC-seq regions of open chromatin.

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.



Conclusions

- SY-1425 drives RARA-high AML cell lines towards more differentiated cell types.
- RARα has a significant role in the gene regulatory circuitry of SY-1425 response.
- Other TFs, such as IRF8, are part of the circuitry of response to SY-1425.
- A biomarker-directed phase 2 trial (NCT02807558) of SY-1425 is currently ongoing in genomically define subsets of AML and MDS patients.