Abstract 2898

Clinical Pharmacodynamic Markers and Combinations with SY-1425 (tamibarotene) in a Genomically-Defined subset of non-APL AML

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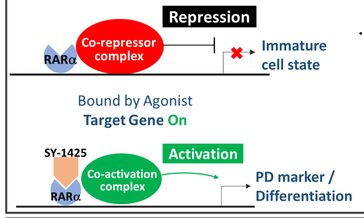
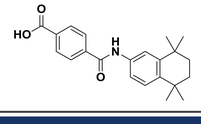
Abstract

SY-1425, a potent and selective agonist of the retinoic acid receptor RARα, is being investigated in a Ph2 trial in a novel genomically-defined subset of AML and MDS patients (clinicaltrials.gov NCT02807558). RARα is a nuclear hormone receptor and transcription factor that regulates genes involved in cell differentiation and proliferation. We identified a super-enhancer (SE) at the *RARA* locus, the gene encoding RARα, in a subset of primary non-APL AML blasts. Preclinical models demonstrated a correlation between the presence of a *RARA* SE and sensitivity to SY-1425, providing the rationale for clinical investigation. Further research has investigated pharmacodynamics (PD) markers and combinations of drugs to support clinical development of SY-1425. In this study we identified *DHRS3* mRNA induction as a measure of RARα target engagement with SY-1425. We also demonstrated synergy in preclinical models with SY-1425 and hypomethylating agents. Since RARα is a transcription factor that regulates target genes when bound by a retinoid, we characterized the dynamic expression changes of a panel of *RARA* enhancer- high and - low non-APL AML cell lines (hereafter referred to as RARA-high and -low) in response to SY-1425 treatment. *DHRS3* showed the largest expression increase following treatment in 3 RARA-high cell lines, with a range of 29 to 115 fold. In contrast, there was a much lower *DHRS3* induction in 3 RARA-low cell lines (range of 1.6 to 6.1 fold). Induction was found to be both time- and dose-dependent with maximal induction around 6 hours and half maximal induction near to the EC50 for the anti-proliferative effect in RARA-high cell lines. *DHRS3* encodes dehydrogenase/reductase (SDR family) member 3, a metabolic enzyme involved in maintaining cellular retinol homeostasis and had previously been shown to be induced by retinoids. Thus, *DHRS3* induction in tumor cells represents a potentially useful PD marker for clinical studies of SY-1425. To better understand the mechanism of induction of *DHRS3* by SY-1425 we examined the chromosomal localization of RARα as well as the epigenomic state of the *DHRS3* locus by ChIP-seq for RARα and H3K27 acetylation, the latter being an indicator of active enhancers and promoters. In the untreated state, OCI-AML3 (a typical RARA-high AML cell line) was found to have multiple RARα binding sites both within and distal to the *DHRS3* gene but minimal H3K27 acetylation. Following treatment with SY-1425, the level of H3K27 acetylation at *DHRS3* increased, resulting in the formation of a SE. Moreover, the SE encompassed the RARα binding sites, consistent with the model in which SY-1425 converts RARα into an activator of *DHRS3* expression. Similar results were seen for the CD38 locus in which SY-1425 treatment increased expression, H3K27 acetylation, and RARα binding. CD38 is a cell surface antigen and marker of myeloid maturation readily analyzed by FACS analysis, suggesting it could be an additional PD marker to be used in clinical studies. Indeed, it was found that SY-1425 induced CD38 cell surface expression at similar levels in RARA-high AML cell lines and the NB-4 APL cell line, but not in RARA-low cell lines. We also investigated combinations of SY-1425 with approved or investigational AML and MDS agents in *in vitro* and *in vivo* models to inform future clinical studies and to further explore potential PD markers unique to the combined action of the drugs. Several standard of care agents and drugs in current development were found to have synergistic interactions with SY-1425 in RARA-high but not RARA-low cell lines. In particular, azacitidine and decitabine each showed strong *in vitro* synergy with SY-1425. Evaluation of SY-1425 plus azacitidine in a RARA-high PDX model of non-APL AML demonstrated a better response compared to either agent alone. Additional genome-wide ChIP-seq and expression studies of RARA-high cells treated with various combinations are being investigated to identify optimal PD markers for these combinations. These studies support the use of *DHRS3* mRNA induction in tumor cells as a PD marker in the recently initiated Ph2 study of SY-1425 in genomically-defined AML and MDS patients (clinicaltrials.gov NCT02807558) and further exploration as a PD marker for future combination studies.

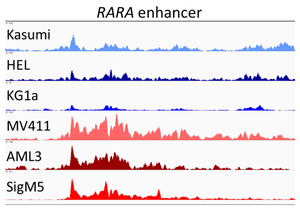
SY-1425 and the treatment of myeloid leukemia

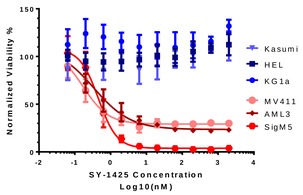


- Developed to overcome liabilities associated with ATRA
  - Very potent and selective for RARα
    - 0.26 nM binding on RARα
    - Greater than 100x selectivity over RARβ and RARγ
  - Not metabolized by Cyp26A1; high sustained blood levels
- Approved (as tamibarotene) in Japan since 2005 for relapsed/refractory APL
  - Over 1400 patients treated
  - Oral drug with well-characterized safety profile
  - High single-agent CR rates in patients who have failed to respond to ATRA
  - Improved CR and molecular CR rates in APL head-to-head studies vs. ATRA



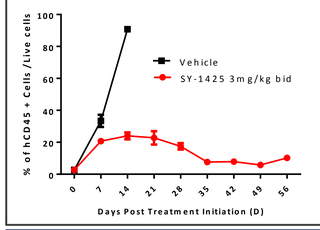
RARA-high predicts for SY-1425 sensitivity

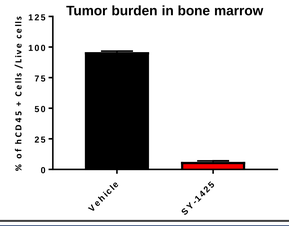




Left: H3K27ac ChIP-seq tracks for the same AML lines with 3 low (blue) and 3 high (red) *RARA* enhancer.
Right: SY-1425 anti-proliferative activity on AML cell lines

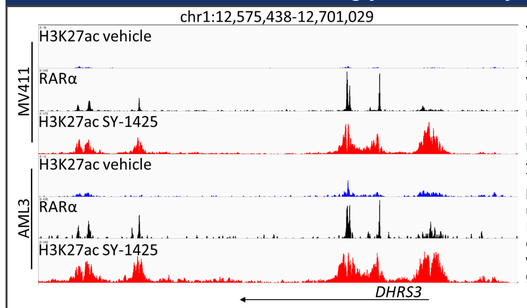
RARA-high PDX models respond to clinically relevant doses of SY-1425



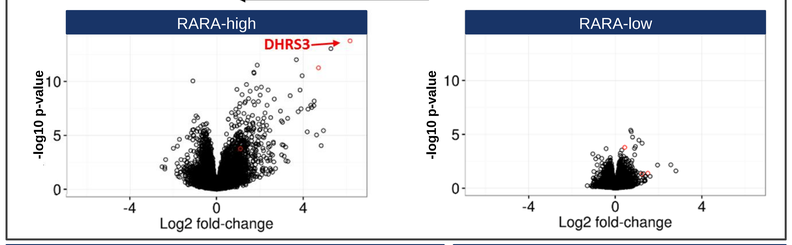


An RARA-high AML PDX model responds to SY-1425 showing reduced peripheral tumor burden and fewer tumor cells in the bone marrow at termination. Mouse dose was chosen to match known human clinical exposure and treatment initiated at 2.7% peripheral burden known to correlate with high initial BM burden.

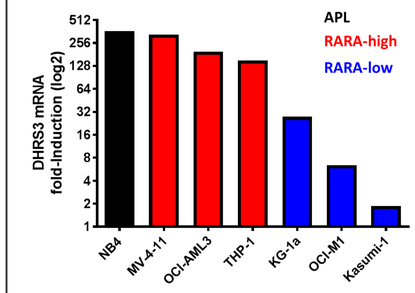
DHRS3 is strongly induced by SY-1425



Top: The gene locus for *DHRS3* shows multiple RARα (black) binding sites in two RARA-high AML cell lines. After vehicle treatment (blue) *DHRS3* has minimal H3K27 acetylation and low initial mRNA expression. After treatment (red) marked increases in acetylation are noted at the promoter and nucleated around RARα binding sites. **Bottom:** Treatment (50nM, 24 hours) of RARA-high AML cell lines causes significant upregulation of RARα target genes. RARA-low cell AML do not have comparable expression changes. DHRS3 was found to be the most upregulated gene by fold change and significance.

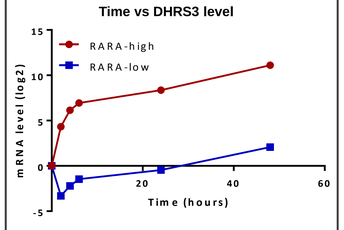


RARA-high DHRS3 induction similar to APL



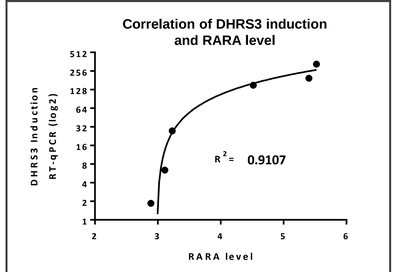
RARA-high AML shows increases in DHRS3 comparable to the NB4 APL cell line harboring the *PML-RARA* oncogenic fusion. RARA-low AML show much lower induction levels

Time dependent induction



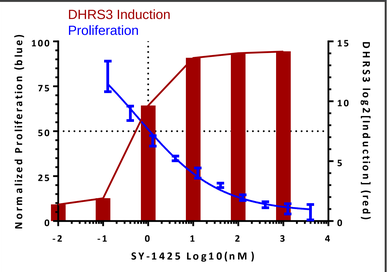
Using qPCR, the rate of induction of DHRS3 was found to rapidly plateau in RARA-high cell line MV411 after 6 hours. Much lower induction in the RARA-low OCI-M1 was observed. This kinetic profile fits clinical feasibility for PD marker sampling post treatment

DHRS3 induction depends on RARA level



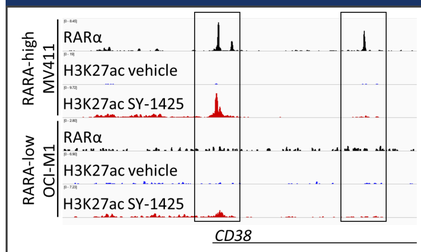
RARA expression level (assessed by RNA-seq) is well correlated with the ability to induce DHRS3 mRNA (assessed by qPCR) after 24h 50nM SY-1425 treatment

DHRS3 induction matches anti-proliferative effect

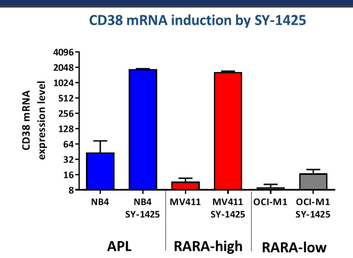


The EC50 of DHRS3 induction was found to occur at a similar concentration to the anti-proliferative IC50 (DMSO normalized) of SY-1425 in an AML cell line model

Maturation gene *CD38* is bound by RARα and induced by SY-1425 in RARA-high cell lines

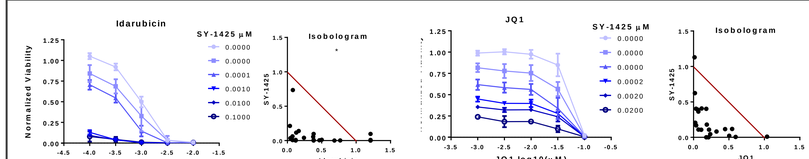


ChIP-seq tracks for RARα (black), H3K27ac vehicle (blue), and H3K27ac post 24 hour SY-1425 treatment (red). Three RARα peaks at the *CD38* locus and increased acetylation post SY-1425 support direct regulation of CD38 expression by RARα.

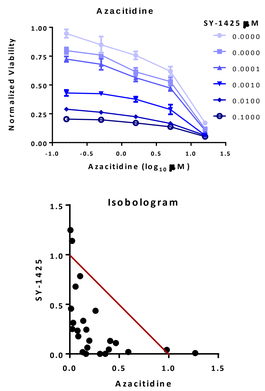


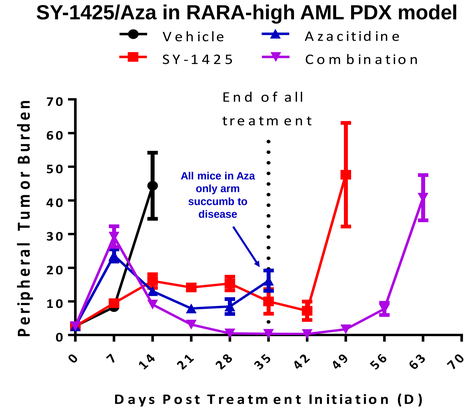
The mRNA expression of CD38 is strongly induced by SY-1425 in both APL (which harbors a *PML-RARA* fusion) and RARA-high AML, but not in AML that has low RARA levels. Similar findings were corroborated by FACS on the same AML models.

SY-1425 shows synergy with SOC and emerging therapies in AML



SY-1425 shows synergistic anti-proliferative effects with both current AML standard of care treatments (**left**, idarubicin) and emerging therapies (**right**, BRD4 inhibitor JQ1) supporting the potential for addition of SY-1425 to multiple combination treatment regimens.





SY-1425 showed synergy with azacitidine *in vitro* in AML cell lines and demonstrated both a deeper reduction in AML tumor cell burden (in weekly sampling of PBMCs) and prolonged time to progress once treatment was discontinued (dotted line) in a RARA-high disseminated AML PDX. The AM5512 model shows incomplete response to SY-1425 monotherapy enabling assessment of combination benefit. Dosing used was SY-1425 3mg/kg BID continuous, azacitidine 2mg/kg QD 7days, or both. Lines for each arm end when all mice on arm succumb due to disease.

Conclusions

- SY-1425 is a first-in-class potent and selective RARα agonist with favorable PK properties and is 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 a strong *RARA* enhancer and increased *RARA* transcript levels
- We have identified target genes of RARα, the induction of which can be implemented as clinical PD markers
  - DHRS3 and CD38 are being measured in an ongoing Phase 2 clinical trial of SY-1425 to get an early assessment of biological activity
- SY-1425 shows synergy with chemotherapy, hypomethylating agents and novel therapies in in AML and MDS models, providing evidence for a clinical combination strategy, in addition to the ongoing single agent strategy
- SY-1425 is currently being investigated in a biomarker-directed Phase 2 trial in genomically defined subsets of AML and MDS patients with high levels of *RARA* gene expression (clinicaltrials.gov, NCT02807558)

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