P6-11-18

San Antonio Breast Cancer Symposium – December 6-10, 2016

A novel subgroup of estrogen receptor positive breast cancer may benefit from Super-Enhancer guided patient selection for retinoic acid receptor α agonist treatment

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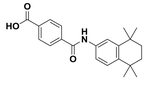
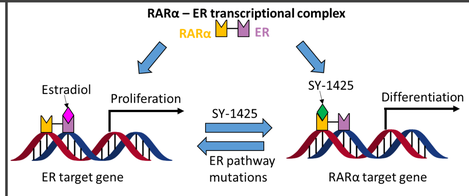
Abstract

Endocrine-resistance remains a major challenge for treatment of breast cancer. Multiple mechanisms for endocrine resistance have been proposed, including altered expression of ER co-regulators such as Retinoic Acid Receptor Alpha (RARα). Furthermore, crosstalk between estradiol and RA signaling is known and upregulation of RARα has been observed in tamoxifen resistance. We propose a novel treatment paradigm for a newly-defined subset of HR+ patients based on our discovery of a super-enhancer (SE) associated with the *RARA* locus. SEs are large, highly active chromatin regions that pinpoint cancer vulnerabilities. The *RARA* SE-identified vulnerability can be targeted using the potent, selective, and metabolically stable RARα agonist SY-1425 (tamibarotene). SY-1425 is approved in Japan to treat Acute Promyelocytic Leukemia, has a well-established efficacy and safety profile, and may enhance response to hormonal therapy (HT) in this newly-defined subset of HR+ patients potentially delaying the need for alternate treatment. Tumor samples from 42 breast cancer patients were analyzed across a range of molecular subtypes. We identified an SE linked to the *RARA* gene in 54.5% of the hormone positive patient samples. *RARA* SEs predicted sensitivity to SY-1425 in 12 breast cancer cell lines confirming their functional role, and showed a correlation with *RARA* gene expression. A panel of 37 breast cancer cell lines was tested for SY-1425 anti-proliferative activity and gene expression levels, and identified *RARA* as the single best predictor of response. Proliferation of RARA-high cells was inhibited by SY-1425 with low nanomolar EC50s. Transcriptional profiling was performed on 4 HR+ and 3 HER2+/HR- breast cancer cell lines and analyzed by GSEA to examine the molecular response to SY-1425. Signatures for growth including E2F, MYC, DNA replication, and cell cycle were significantly downregulated while retinol metabolism and luminal signaling were upregulated. Estrogen signaling was also significantly altered by SY-1425, supporting known crosstalk between RARα and ER. Consistent with differentiation, CYP26A1 and VE-Cadherin were induced and Actin and Ki67 were diminished at relevant concentrations of SY-1425 and could serve as pharmacodynamic markers of response. To test responses to SY-1425 *in vivo*, two cell line-derived models and two patient-derived breast cancer models (one RARA-high, and one RARA-low each) were treated with SY-1425. SY-1425 inhibited tumor growth in the RARA-high models, but not the RARA-low models (43% versus 0% TGI). Consistent with the observed changes in transcription, SY-1425 in combination with tamoxifen synergistically inhibited proliferation of RARA-high breast cancer cell lines. Although a few clinical studies have investigated the use of ATRA in HR+ breast cancer without success, our results suggest that patient selection based on the *RARA* SE may predict which HR+ breast cancer patients could derive benefit by adding an RARα agonist to HT. The potential to prolong or increase the clinical effect of anti-estrogen therapy with SY-1425, which has improved potency, selectivity, and PK stability versus ATRA, would be an attractive strategy to explore.

About SY-1425 and a proposed model

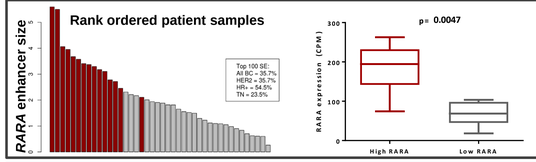
- Developed to overcome liabilities associated with ATRA
  - Very potent and selective for RARα
    - 0.26 nM binding on RARα
    - Greater than 100x selectivity over RARβ and RARγ
    - No activity outside of RAR family
  - Not metabolized by Cyp26A1; high sustained blood levels
- Approved (as tamibarotene) 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





- We hypothesized that SE analysis and SY-1425 could be used together to identify and treat a subset of breast cancer patients
- RARα and ER have previously been shown to have signaling cross talk in breast cancer with RARα elevated in hormone therapy relapsed patients
- ATRA has been used *in vitro* to probe this biology but has limited translatability in this indication due to lower potency, selectivity, exposure, and dosing stability compared to SY-1425

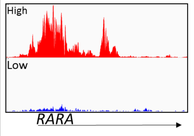
RARA is associated with an SE in Breast Cancer

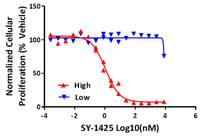


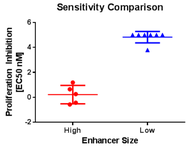
A cohort of 42 primary breast cancer samples was profiled for their genome-wide distribution of H3K27ac, a marker of enhancers, by ChIP-seq. The waterfall plot shows the distribution of the *RARA* enhancer across the samples. Patient samples with *RARA* among the 100 largest enhancers are highlighted in red. This patient subset is primarily HR+ and shows significant upregulation of RARα mRNA.

RARA associated SE predicts for response to SY-1425

1. SE defines novel subtype in breast cancer
2. SY-1425 effect is highly dependent on SE level
3. SE high or low distinctly defines responders and non-responders

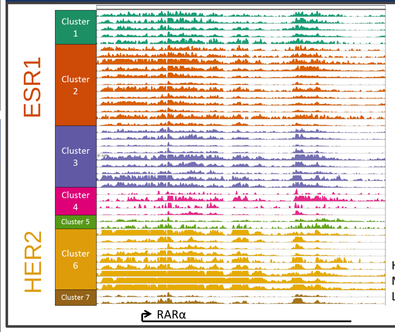


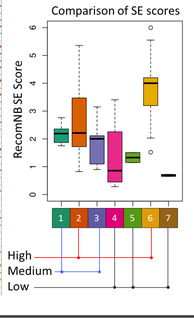




An SE at *RARA* is highly differential among breast cancer patients samples and models. The presence of the SE predicts for anti-proliferative response. Moreover, the size of the enhancer can be used to predict sensitive and insensitive breast cancer models.

Patient epigenomic profiling shows RARA association with HER2 and ESR1





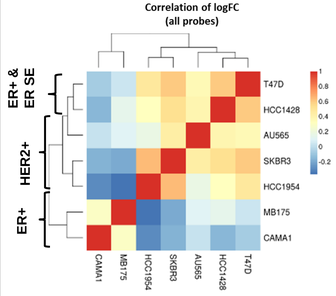
Using the distributions of enhancers in a cohort of patient samples of mixed molecular type, the enhancer at *RARA* is found to be strongest in two particular clusters:

Cluster 2 which is associated with large ESR1 SEs;

Cluster 6 is associated with ERBB2/HER2 SEs.

Both findings are in agreement with historical literature showing that RARα cooperates with estrogen receptor and can get co-amplified with the *HER2* locus

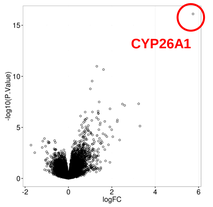
SY-1425 impairs cancer growth pathways



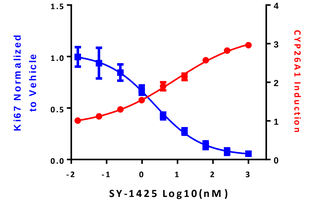
Gene set	NES	FDR
HALLMARK_MYC_TARGETS_V1	-2.764	<0.001
HALLMARK_E2F_TARGETS	-2.629	<0.001
REACTOME_MITOTIC_PROMETAPHASE	-2.497	<0.001
REACTOME_DNA_REPLICATION	-2.416	<0.001
REACTOME_G2_M_CHECKPOINTS	-2.362	<0.001
REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM	-2.351	<0.001

Seven RARA-breast cancer cell lines that showed anti-proliferative response to SY-1425 were profiled for genes changes. Overall, the response to SY-1425 depended on their original hormone or HER2 status based on clustering of the correlated mRNA level changes. However, significant downregulation of growth pathways including E2F and MYC was conserved among all seven.

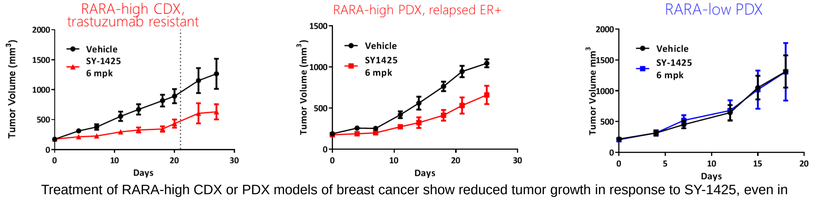
Gene expression changes identify PD markers



To confirm downstream effect of SY-1425 we sought a potential PD marker of SY-1425 response that would be conserved between breast cancers of different origins. **Left:** we examined the average gene changes across all seven lines. **Right:** CYP26A1, a RARα target gene and retinoic acid metabolic gene was profoundly upregulated. Protein level changes for CYP26A1 (red, induced) and proliferative capacity with Ki67 (blue, reduced) were verified by high-content imaging in a RARA-high cell line.

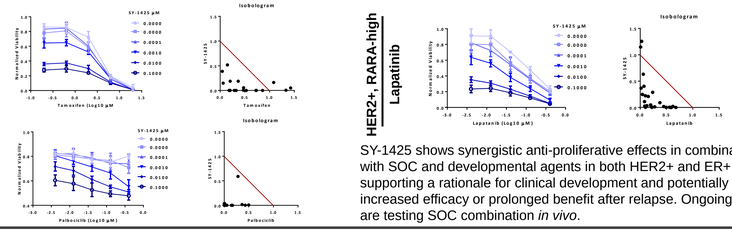


SY-1425 demonstrates tumor growth reduction in RARA-high BC models



Treatment of RARA-high CDX or PDX models of breast cancer show reduced tumor growth in response to SY-1425, even in trastuzumab and hormone resistant models, while RARA-low models (PDX shown in blue) do not respond to treatment with SY-1425

SY-1425 shows synergy with standard of care agents in BC



SY-1425 shows synergistic anti-proliferative effects in combination with SOC and developmental agents in both HER2+ and ER+ models supporting a rationale for clinical development and potentially increased efficacy or prolonged benefit after relapse. Ongoing studies are testing SOC combination *in vivo*.

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
- Super-enhancer analysis identify a subset of breast cancer patient tumors that may have a unique dependency on RARα
  - SY-1425 induces an anti-proliferative response in *RARA*-high breast cancer cell lines as well as CDX and PDX models breast cancer
- SY-1425 down-regulates genes associated with tumor growth pathways in HER2+ and ER+ models
- SY-1425, in combination with anti-ER and anti-HER2 agents, shows synergistic anti-tumor effects that are being tested in xenograft models of *RARA*-high breast cancer
- The use of a patient-selection biomarker and improved features of SY-1425 may overcome limitations of ATRA, a non-selective retinoic acid agonist, as revealed in previous breast cancer studies
- SY-1425 is currently being investigated in a biomarker-directed Phase 2 trial of genomically defined subsets of AML and MDS (clinicaltrials.gov, NCT02807558)