Abstract P188

Mechanistically informed combinations of SY-1425, a potent and selective RARα agonist, with hypomethylating or anti-CD38 targeted agents in AML and MDS

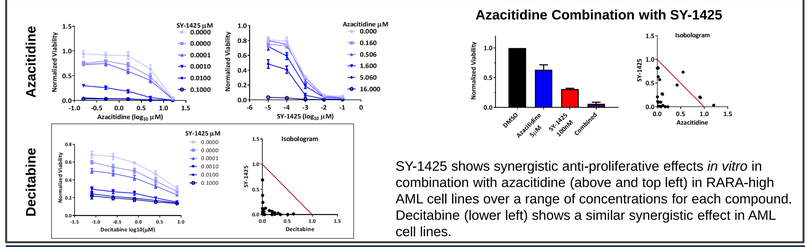
Michael R McKeown, Kathryn Austgen, Chris Fiore, Emily Lee, Darren Smith, Christian Fritz, Tracey Lodie, Emmanuelle di Tomaso, Eric Olson

Syros Pharmaceuticals, 620 Memorial Drive, Cambridge, MA 02139

Abstract

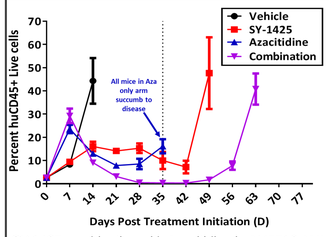
Background: The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tamibarotene) and other agents to build on the monotherapy strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARα mediated myeloid gene activation of SY-1425, epigenetic priming with hypomethylating agents (HMAs) and CD38 induction were explored. **Aims:** We sought to investigate mechanistically informed combinations of SY-1425 with standard of care agents and with potential novel agents in AML. We hypothesized that the HMA azacitidine could prime AML cells for SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA). **Methods:** HMA synergy was tested *in vitro* in AML cell lines over a range of concentrations for SY-1425 and azacitidine. *In vivo* studies used a disseminated patient derived xenograft (PDX) model of AML expressing high levels of RARA. SY-1425 induction of CD38 was assessed by H3K27ac ChIP-seq, RARα ChIP-seq, RNA-seq and flow cytometry. Antibody dependent cell-mediated cytotoxicity (ADCC) was tested in an *ex vivo* co-culture model of human NK cells and AML cell lines. **Results:** RARα acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDX demonstrated superior reduction of tumor burden (< 1% detectable tumor cells) vs either treatment alone (7% with SY-1425 and 8% with azacitidine). Various combination regimens evaluated in the PDX model over two cycles (56 days) found that 1 week of azacitidine followed by 3 weeks of SY-1425 maximized for anti-tumor activity (< 5% AML cells in periphery, bone marrow and spleen) and tolerability (< 8% weight loss). RARα binds directly to the *CD38* locus and induces H3K27 acetylation in response to SY-1425 causing CD38 to be one of the most upregulated mRNA transcripts in RARA-high models. SY-1425 treatment of four RARA-high AML cell lines and three RARA-high primary AML patient samples induced cell surface CD38 to high levels comparable to those of DARA sensitive multiple myeloma cells. In contrast, no CD38 induction was observed in RARA-low cell lines. RARA-high AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent controls and exhibited a 5-10 fold increase in NK cell-dependent activation measured by IFNγ secretion.
Summary/Conclusion: The RARA biomarker dependent synergy with azacitidine and SY-1425 is hypothesized to work through hypomethylation based priming of myeloid differentiation by SY-1425 agonism of formerly repressed RARα target genes. Since CD38 is one of the most strongly induced RARα target genes in response to SY-1425, AML blasts can be sensitized to DARA in a biomarker dependent manner. The preclinical synergistic effects and anticipated non-overlapping clinical toxicity profiles of the respective agents provide a strong rationale for clinical evaluation of each SY-1425 combination in biomarker selected AML and MDS patients.

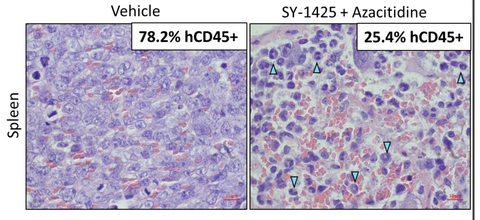
SY-1425 synergizes with HMAs in RARA-high AML cell lines *in vitro*



SY-1425 shows synergistic anti-proliferative effects *in vitro* in combination with azacitidine (above and top left) in RARA-high AML cell lines over a range of concentrations for each compound. Decitabine (lower left) shows a similar synergistic effect in AML cell lines.

Combination shows increased depth and duration of response in PDX

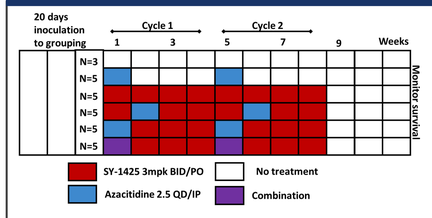


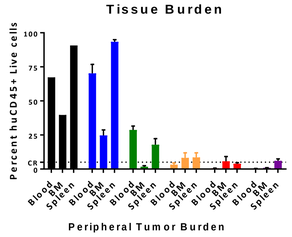


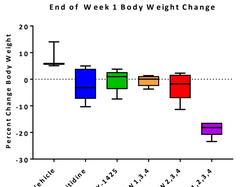
SY-1425 combination with Azacitidine in AM5512 AML PDX model. Treatments started 20 days after inoculation of mice with human tumor cells. Percent of human CD45+ cells in peripheral blood sampled weekly. Arms terminate when all mice on arm lost. Dashed line indicates end of treatment for all arms

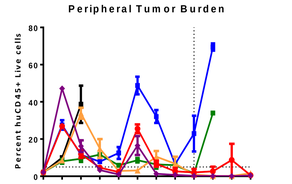
Tumor burden in tissues is decreased by combination with evidence of differentiation at two weeks. Increased cell diversity, and decreased blasts and cellularity are seen combination treatment. Cyan arrows mark some examples of cells present with myeloid differentiation morphology. Percentage based on flow cytometry of sample.

Combination with azacitidine optimized for response and tolerability



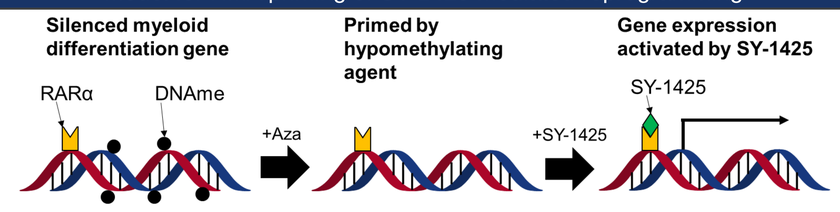




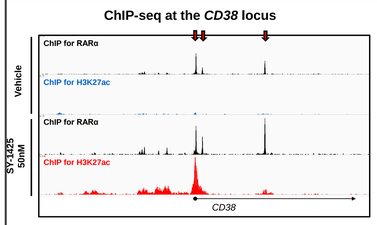


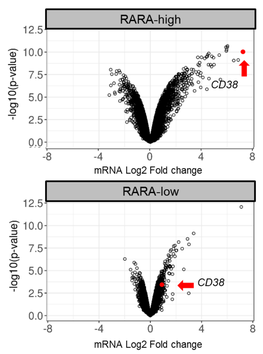
Top, left: Design of study with single agents and three combination schedules
Center: Color legend for subsequent figures
Bottom, left: End of first week showed most body weight loss on treatment, particularly for the arm co-administered during W1
Top, right: Consistent with peripheral burden, Aza week 1 combinations showed strongest reductions in tissue tumor burden dropping below 5% for mice receiving two cycles
Bottom, right: Peripheral tumor burden by huCD45+ FACS shows strongest reduction with Azacitidine week 1 combinations and best survival. Treatment started on day 21 post inoculation. Vertical dashed line indicates end of treatment

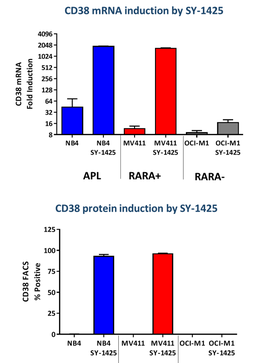
Model for HMA priming of SY-1425 mediated reprogramming



CD38, a marker of myeloid cell maturation, is a direct target gene of RARα, leading to targeted upregulation by SY-1425

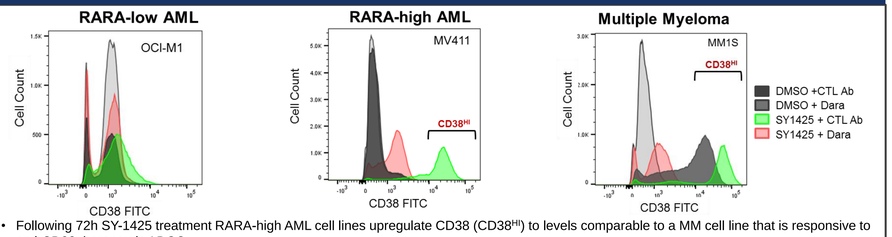






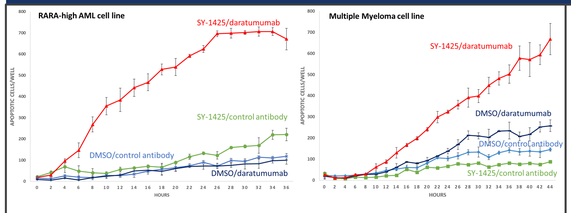
RARα is directly bound to the *CD38* locus as shown **(left panel)** through chromatin IP of RARα itself in a RARA-high cell line. Red arrows indicate the RARα binding sites. SY-1425 induces H3K27ac deposition at the *CD38* locus indicative of transcriptional activation. CD38 is one of the most upregulated genes in RARA-high **(middle panels)** and is upregulated at both the mRNA and protein level **(right panels)** in RARA-high similar to PML-RARA fusion driven APL but not in RARA-low

SY-1425 drives expression of *CD38* in RARA-high AML cells to levels comparable to Multiple Myeloma

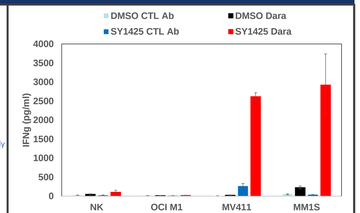


- Following 72h SY-1425 treatment RARA-high AML cell lines upregulate CD38 ($CD38^{HI}$) to levels comparable to a MM cell line that is responsive to anti-CD38 therapeutic ADCC.
- Daratumumab (Dara) efficiently blocks SY1425-induced CD38 in RARA-high AML cell lines (lighter grey) indicating complete binding
- In contrast, a RARA low AML cell line demonstrates no significant change in CD38.

Combination of SY-1425 and Daratumumab led to apoptosis of tumor cells and immune cell activation

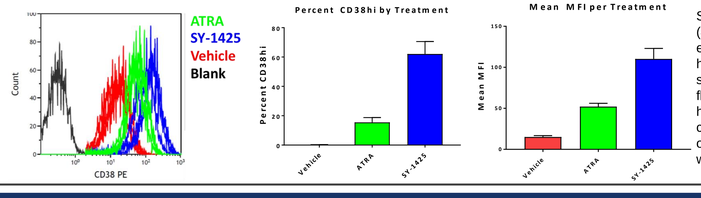


Quantification of apoptotic (AnnexinV+) cells/well during 72h co-culture time course of NK cells and RARA high AML cell line or MM cell line demonstrates robust ADCC of the AML cell line to levels comparable to a multiple myeloma cell line.



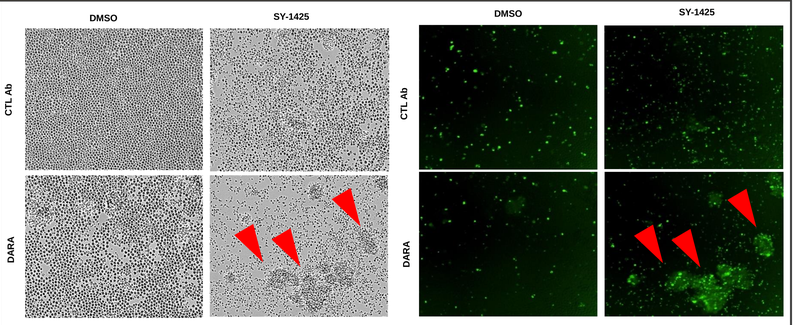
IFNγ secretion from NK cells is strongly induced in the combination treatment of the NK and RARA high AML cell line co-culture and multiple myeloma cell line. No IFNγ is observed in the RARA low co-culture.

SY-1425 shows higher CD38 induction than ATRA in a CDX model of AML



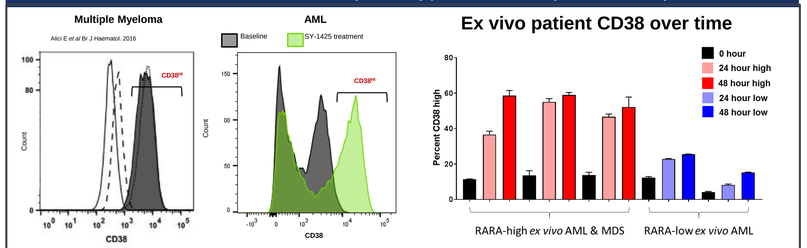
SY-1425 (3mpk BID) and ATRA (4mpk BID) were dosed at exposures matched to known human PK. SY-1425 caused a shift toward higher CD38 fluorescence intensity and a higher percentage of CD38hi cells. This effect was found at one, two, and three (shown) weeks of treatment.

Combination SY-1425 and anti-CD38 therapeutic antibody induces NK cell dependent ADCC



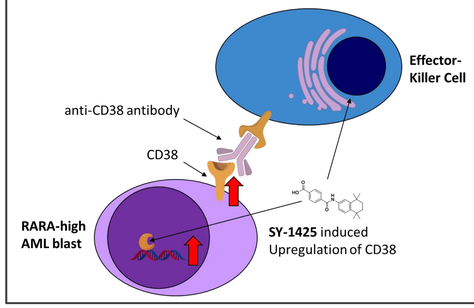
Human NK cells were co-cultured with a RARA-high AML cell line for 72h following treatment and ADCC and NK cell activation were monitored by microscopy for morphology (phase contrast) and AnnexinV staining of tumor cells. Red arrows indicate activated NK cells and AnnexinV staining of tumor cells showing appreciable cell death in context of combination treatment only.

SY-1425 induces $CD38^{Hi}$ phenotype in *ex vivo* patient samples



- SY-1425 induces a $CD38^{HI}$ phenotype in primary AML patient samples (commercially obtained) similar to what is observed in an anti-CD38 therapeutic antibody sensitive multiple myeloma patient **(left)**.
- In addition, the $CD38^{HI}$ phenotype is only observed in RARA-high *ex vivo* AML patient samples treated with SY-1425 **(right).**

Proposed mechanism of SY-1425 and anti-CD38 antibodies



- AML blast cells are naturally CD38 negative or dim which is inadequate for a robust anti-CD38 mediated response
- SY-1425 leads to an upregulation of CD38 protein expression on the surface of AML cells
- May also reduce immunosuppression and promote immune activation response

Conclusions

- SY-1425, an oral and selective RARα agonist, induces differentiation in RARA-high cell lines and patient samples but not in RARA-low.
  - Currently approved in Japan for the treatment of relapsed refractory APL
  - Cancer pathogenesis is complex and often optimally treated through multiple combined mechanisms
  - By identifying alterations in gene regulation, Syros' platform uncovers de novo liabilities for tumor cells leading to novel targeted combinations with therapeutic potential
- Hypomethylating agents identified as promising combination agents with SY-1425
  - Synergy seen in RARA-high AML cell line and *in vivo* models
  - Synergy based on complementary gene activation and differentiating mechanisms of the respective drugs
- AML PDX studies identified a regimen to maximize tumor suppression and tolerability, supporting a clinical combination strategy
- SY-1425 is being investigated as a monotherapy and in combination with azacitidine in a biomarker-directed Phase 2 trial in biomarker defined subsets of AML and MDS patients (clinicaltrials.gov, NCT02807558)
- CD38, a marker of maturation, is upregulated in response to SY-1425
  - The *CD38* gene is a direct target of RARα leading to potent and specific upregulation
  - SY-1425 induces $CD38^{HI}$ expression in biomarker-high AML cells at a level comparable to MM
  - CD38 induced AML cells become more sensitive to daratumumab, a known effective agent in MM
- SY-1425 + daratumumab combination is more active than either single agent in AML models.
- This data supports future clinical exploration of the combination of SY-1425 and daratumumab in RARA biomarker positive AML patients.

EHA Annual Meeting 2017