Abstract 3085

SY-1425 (tamibarotene), a selective RARα agonist, shows synergistic anti-tumor activity with hypomethylating agents in a biomarker selected subset of AML

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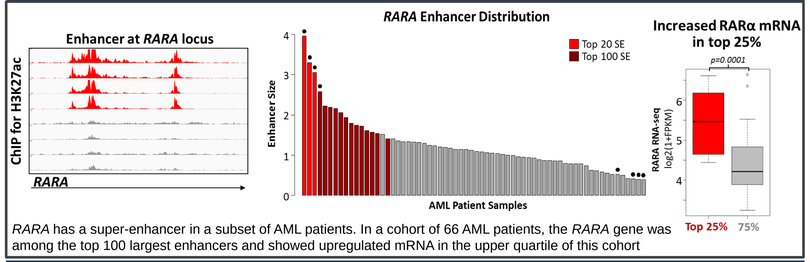
Abstract

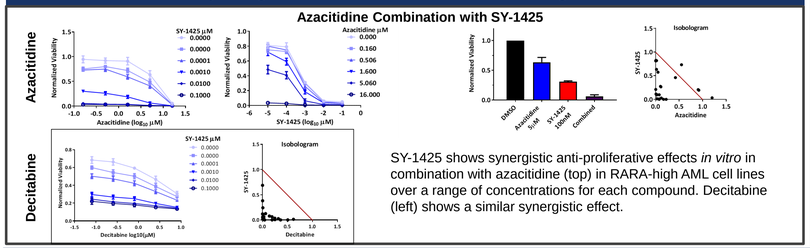
In patients with acute myeloid leukemia (AML) (≥ 60 years) and myelodysplastic syndrome (MDS), the use of hypomethylating agents (HMAs) may extend survival, but cure rates are very low and new treatment approaches are needed. HMAs, such as azacitidine, work by inhibiting DNMT1, leading to depletion of DNA methylation in the tumor cells. Hypomethylation, in turn, leads to the re-expression of genes associated with differentiation and growth arrest. We have recently explored the potent and selective RARα agonist SY-1425 in a genomically defined subset of AML. SY-1425 binds to RARα and causes a transition from repression to strong activation of target genes, thus reprogramming the tumor cells toward terminal maturation in RARA-high AML models, supporting our recently initiated Phase 2 trial in a biomarker-selected subset of AML and MDS (NCT02807558). Based on potential mechanistic synergy, we evaluated SY-1425 in combination with HMAs and identified a synergistic anti-proliferative effect. In RARA-high AML cell lines, but not RARA-low, the combination of SY-1425 with either azacitidine or decitabine showed synergistic anti-proliferative effects on the cells, with combination indices less than 0.5 over a range of concentrations from 0.01 to 100nM of SY-1425 and 0.1 to 1μM of HMAs. SY-1425 and azacitidine were also co-administered to a disseminated patient-derived xenograft (PDX) mouse model of RARA-high AML. The combination demonstrated superior reduction of tumor burden vs either therapy alone, leading to deeper and more durable responses with less than 1% detectable tumor burden. A follow-up study in the RARA-high PDX model investigated different treatment schedules of SY-1425 and azacitidine over a period of 56 days, supporting a regimen that maximizes anti-tumor activity and tolerability. Mechanistic studies using RNA-seq and ChIP-seq in AML cell line models have revealed that while azacitidine had only moderate suppressive or activating effects over a broad set of genes, the addition of SY-1425 in RARA-high models resulted in strong and specific induction of genes bound by RARα. It is hypothesized that azacitidine acts to prime the tumor cells for reprogramming by SY-1425. The loss of methyl-cytosine residues following azacitidine treatment lowers the barrier to SY-1425 mediated gene induction. It was observed that the two agents work cooperatively to promote terminal differentiation and decrease proliferation of the AML tumor cells, with the potential for increased clinical benefit in a subset of AML defined by a *RARA* super-enhancer. Based on the largely non-overlapping clinical toxicity profiles of azacitidine and SY-1425, supported by the observed tolerability of the combination in preclinical models, these findings provide a strong rationale for a planned study of this combination in biomarker selected, newly diagnosed AML patients.

RARA Expression is Driven by a Super-Enhancer in a Subset of AML



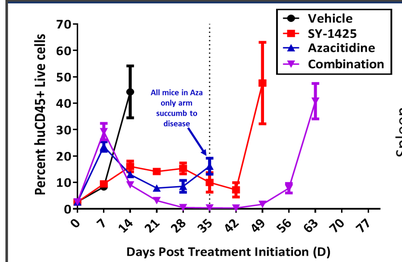
RARA has a super-enhancer in a subset of AML patients. In a cohort of 66 AML patients, the *RARA* gene was among the top 100 largest enhancers and showed upregulated mRNA in the upper quartile of this cohort

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

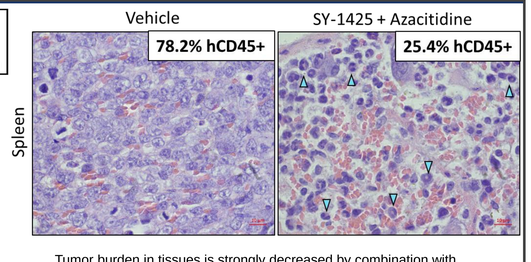


SY-1425 shows synergistic anti-proliferative effects *in vitro* in combination with azacitidine (top) in RARA-high AML cell lines over a range of concentrations for each compound. Decitabine (left) shows a similar synergistic effect.

Combination Shows Better Depth and Duration of Response in PDX

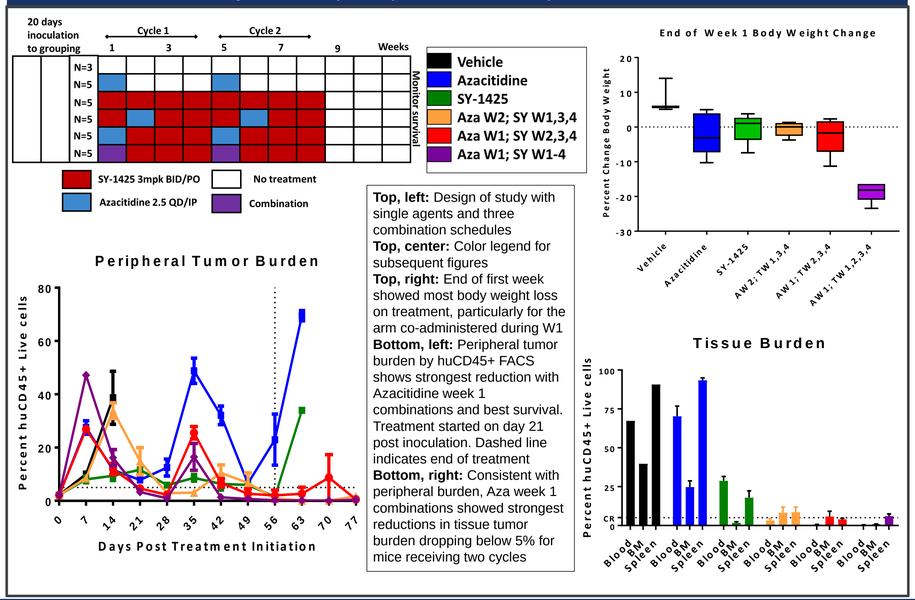


SY-1425 combination with Azacitidine in AM5512 AML PDX model. Treatments started 20 days after inoculation of mice with human tumor cells. Percent of human CD45+ cells in peripheral blood sampled weekly. Arms terminate when all mice on arm lost. Dashed line indicates end of treatment for all arms



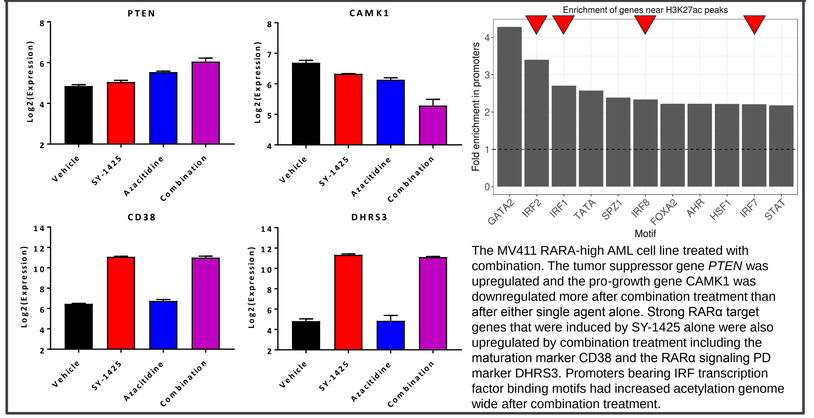
Tumor burden in tissues is strongly decreased by combination with evidence of differentiation at two weeks. Increased cell diversity, and decreased blasts and cellularity are seen in combination. Cyan arrows mark some examples of cells present with myeloid differentiation morphology. Percentage based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

Combination Regimen Study to Optimize Tolerability and Tumor Burden Reduction



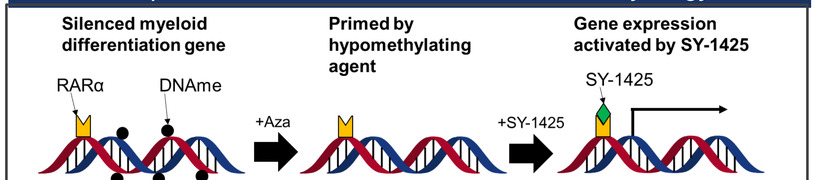
Top, left: Design of study with single agents and three combination schedules
Top, center: Color legend for subsequent figures
Top, right: End of first week showed most body weight loss on treatment, particularly for the arm co-administered during W1
Bottom, left: Peripheral tumor burden by huCD45+ FACS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

Combination Enhances Gene Regulatory Effect



The MV411 RARA-high AML cell line treated with combination. The tumor suppressor gene *PTEN* was upregulated and the pro-growth gene CAMK1 was downregulated more after combination treatment than after either single agent alone. Strong RARα target genes that were induced by SY-1425 alone were also upregulated by combination treatment including the maturation marker CD38 and the RARα signaling PD marker DHRS3. Promoters bearing IRF transcription factor binding motifs had increased acetylation genome wide after combination treatment.

Proposed Mechanism of SY-1425 & HMA Synergy



DNA methylated by aberrant DNMT1 activity and RARα unbound by ligand leads to repression

Inhibition of DNMT1 depletes methylation from DNA and primes for RARα mediated reprograming

SY-1425 binds RARα switching it to a potent gene activator; upregulation of myeloid genes

Conclusions

- SY-1425, a potent and selective oral RARα agonist (currently approved in Japan for the treatment of relapsed refractory APL) shows synergy with hypomethylating agents
  - Synergy seen in RARA-high AML cell line and *in vivo* models but not in RARA-low
  - 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
- Cancer pathogenesis is complex and often optimally treated through multiple combined mechanisms
- 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)

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