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SY-1425, a selective RARα agonist, induces high levels of CD38 expression in RARA-high AML tumors creating a susceptibility to anti-CD38 therapeutic antibody treatment.

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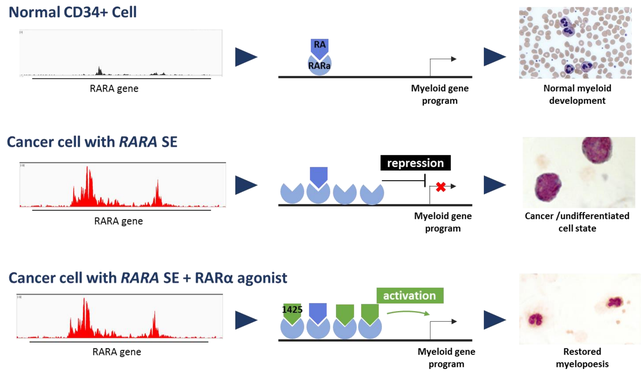
Abstract

CD38 is a cell surface protein expressed primarily on white blood cells and considered a marker of differentiation initiation. CD38 is involved in the immune system by engaging cross-talk with T and B cells as well as activation of NK cells. In multiple myeloma (MM), a subset of tumor cells have high CD38 expression ($CD38^{hi}$), which has led to the development of effective anti-CD38 therapeutic antibodies, such as daratumumab (DARA). Thus, cancer cells that express CD38 can be selectively targeted for elimination by the immune system using these therapeutic antibodies. In multiple myeloma, DARA is most effective in patients whose tumor cells are $CD38^{hi}$. In contrast, CD38 expression in AML tumors is generally observed to be negative ($CD38^{neg}$) or dim ($CD38^{dim}$), and DARA has not shown activity in preclinical AML models. We previously reported that SY-1425, a clinical stage RARα agonist with improved pharmacokinetics, potency, and selectivity over pan-retinoic acid agonists, induces differentiation in non-APL AML cell lines and primary patient samples with a *RARA* super-enhancer associated biomarker (RARA-high). Since CD38 was found to be among the most differentially expressed genes in response to SY-1425, we hypothesized that SY-1425 mediated CD38 induction to levels comparable to MM may sensitize RARA-high AML cells to anti-CD38 therapy.

We demonstrate that SY-1425 treatment of four RARA-high AML cell lines and four RARA-high primary AML patient PBMCs induces the $CD38^{hi}$ phenotype, as measured by flow cytometry, similar to that found in the DARA sensitive MM cells. In contrast, we see no induction in RARA-low cell lines. We then demonstrated the activity of the SY-1425 and DARA combination in an *ex vivo* NK cell co-culture assay. Two RARA-high AML cell lines treated with SY-1425 and DARA were co-cultured with NK cells and monitored for both antibody dependent cell-mediated cytotoxicity (ADCC) and NK cell activation by interferon gamma production. The combination of SY-1425 and DARA led to a six-fold increase in tumor cell death relative to the single agent controls, and 5-10 fold increases in NK cell activation is observed only in the SY-1425 and DARA combination treatment of RARA-high AML cell lines. Neither single agent, when administered alone, resulted in ADCC. Furthermore, a RARA-low AML cell line does not respond in the ADCC assay following the combination treatment due to the lack of CD38 induction in this SY-1425 insensitive line.

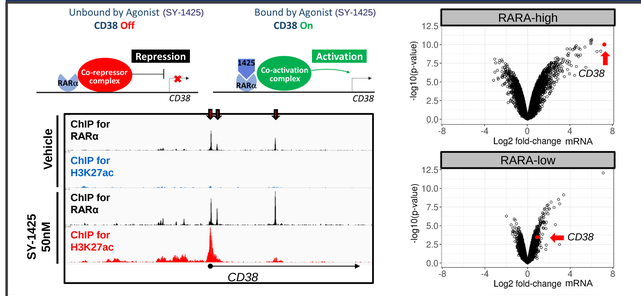
In summary, we have identified a novel and rational combination treatment approach for a subset of patients with RARA-high AML. By inducing the expression of CD38, SY-1425 in combination with DARA elicits tumor cell death and NK cell activation. Based on these findings, a phase 2 clinical study with SY-1425 in combination with an anti-CD38 antibody is planned in AML using an RARA biomarker patient selection strategy.

SY-1425 activates differentiation through RARα target genes in AML cell lines



In normal myeloid development, the binding of retinoic acid to RARα directly induces the myeloid gene program. However, high expression of the RARα gene, indicated through the presence of a super enhancer (H3K27ac), results in repression of the normal myeloid differentiation pathway. SY-1425, a RARα agonist restores myelopoiesis and induces differentiation of AML blasts.

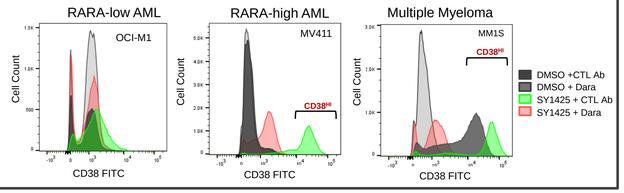
CD38, a marker of myeloid cell maturation, is a direct target gene of RARα, leading to strong targeted upregulation by SY-1425



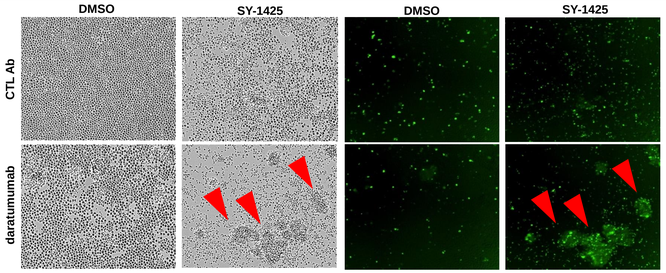
RARα is directly bound to the *CD38* locus as shown (**left panel**) through chromatin IP of RARα in a RARA-high cell line. Furthermore, following SY-1425 treatment, high levels of H3K27ac are induced at the *CD38* locus, indicating transcriptional activation. In support of this (**right panel**) *CD38* is one of the most highly upregulated genes in RARA-high AML cell line following SY-1425 treatment.

SY-1425 drives expression of *CD38* in RARA-high AML cells to levels comparable to a MM cell line that is responsive to anti-CD38 therapeutic antibody-mediated ADCC

- 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.
- In contrast, a RARA low AML cell line demonstrates no change in CD38.

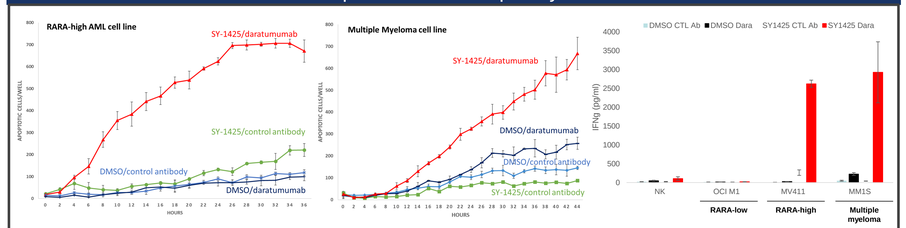


Combination SY-1425 and anti-CD38 therapeutic antibody (daratumumab) treatment of a RARA-high AML cell line induces robust ADCC in a NK cell co-culture



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.

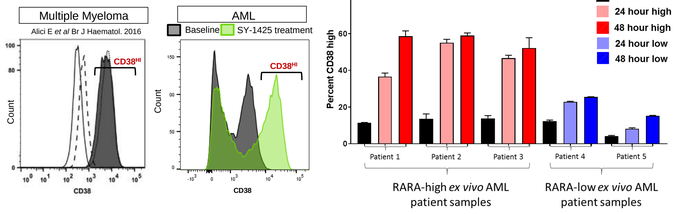
SY-1425 + daratumumab combination induces potent ADCC in a RARA-high AML cell line and to levels comparable to a multiple myeloma cell line



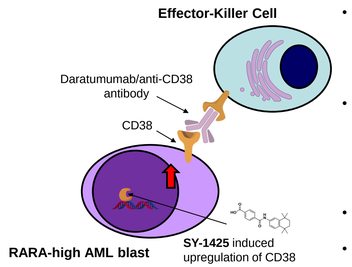
Left and middle panels: Quantification of apoptotic (AnnexinV+) cells/well during 72h co-culture time course of NK cells and RARA high AML cell line demonstrates robust ADCC of the AML cell line to levels comparable to a multiple myeloma cell line. **Right panel**: IFNgamma secretion from NK cells is strongly induced in the combination treatment of the NK and RARA high AML cell line co-culture and multiple myeloma cell line. No IFNgamma is observed in the RARA low co-culture. **Note:** Quantification of apoptotic cells/well during co-culture of NK cell and RARA low AML cell line depict clear insensitivity of this cell line to the combination treatment.

SY-1425 induces $CD38^{Hi}$ phenotype in AML patients which is comparable to Daratumumab sensitive MM 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).**



Conclusions



- By identifying alterations in gene regulation, Syros' platform uncovers de novo liabilities for tumor cells leading to novel targeted combinations with therapeutic potential.SY-1425, an oral and selective RARα agonist, induces differentiation in RARA-high cell lines and patient samples but not in RARA-low.
- *CD38,* a marker of differentiation, is a direct target of RARα and which leads to robust and selective CD38 upregulation upon treatment with SY-1425.
  - 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.

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