CD38 is a cell-surface protein expressed primarily on white blood cells and considered a marker of differentiation. CD38 is involved in the immune system by engaging cross-link with T and B cells as well as activation of NK cells. In multiple myeloma, CD38 has been used for the development of effective anti-CD38 therapeutic antibodies, such as daratumumab (DARA). However, cancer cells that express CD38 can be selectively targeted by the immune system using these therapeutic antibodies as a biomarker. In multiple myeloma, DARAs is most effective in patients whose tumor cells are CD38+ as measured by flow cytometry, similar to that found in the DARA locus as shown (Fig. S1). NK cells and CD38 expression in biomarker locus, AML patient samples DARA secretion from NK cells is in RARA vehicle staining SY1425 Dara for E multiple myeloma, DARA is most effective in patients whose tumor cells are CD38+ by inducing the expression of CD38, such as daratumumab (DARA). Thus, cancer cells that are CD38+ are more susceptible to anti-CD38 therapeutic antibodies. CD38 is involved in the immune system by engaging cross-link with T and B cells as well as activation of NK cells. In normal myeloid development, the binding of retinoic acid to RARα directly induces the myeloid gene program. However, high levels of CD38 expression on individual AML cell lines and primary patient samples with a RARA+ super-enhancer associated biomarker (RARA-High). CD38 is found to be among the most highly expressed genes in response to a RARA-mediated CD38 induction to levels comparable to MM may sensitize RARA-high AML cells to anti-CD38 therapy. We demonstrated that SY1425 treatment of four RARA-high AML cell lines and four RARA-high primary AML patient PBMCs induces the CD38+ 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 SY1425 and DARA combination in an ex vivo NK cell co-culture assay. Two RARA-high AML cell lines treated with SY1425 and DARA were more double stained (CD38+) and monitored for both antibody-dependent cell-mediated cytotoxicity (ADCC) and NK cell activation by interferon gamma production. The combination of SY1425 and DARA led to a significant increase in tumor cell death relative to the single agent controls, and 5-10 fold increases in IFNgamma production is observed in the SY1425 and DARA combination treatment of RARA-high AML cell lines. Neither SY1425 nor DARA induced CD38+ in RARA-low AML cells. Therefore, SY1425 treatment of four RARA-high AML cell lines and primary patient samples with a RARA+ super-enhancer associated biomarker (RARA-High). CD38 is found to be among the most highly expressed genes in response to a RARA-mediated CD38 induction to levels comparable to MM may sensitize RARA-high AML cells to anti-CD38 therapy. SY1425 drives expression of CD38 in RARA-AHL AML cells to levels comparable to a MM cell that is responsive to anti-CD38 therapeutic antibody-mediated ADCC. SY1425 + daratumumab combination induces potent ADCC in a RARA-high AML cell line and to levels comparable to a multiple myeloma cell line.

**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.**

SY1425 activates differentiation through RARα target genes in AML cell lines

**CD38, a marker of myeloid cell maturation, is a direct target gene of RARα, leading to strong targeted upregulation by SY1425.**

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

**SY1425 + daratumumab combination induces potent ADCC in a RARA-high AML cell line and to levels comparable to a multiple myeloma cell line.**

**Conclusions**

- By identifying alterations in gene regulation, Syros’ platform uncovers new novel liabilities for tumor cells leading to novel targeted combinations with therapeutic potential. SY1425, an oral and selective RARα agonist, induces differentiation in RARA high cell lines and patient samples but not in RARA- low cell lines.

- CD38 is a biomarker of differentiation, and a direct target of RARαs and which leads to robust and selective CD38 expression, and ADCC treatment shown to induce differentiation in RARA high cell lines and patient samples.

- SY1425 induces CD38+ expression in biomarker high AML cell lines most comparable to MM.

- CD38 induced AML cells become more sensitive to daratumumab, a human monoclonal antibody against RARα.

- SY1425 + daratumumab combination is more active than either single agent in AML models.

- This data supports future clinical exploration of the combination of SY1425 and daratumumab in RARA biomarker positive AML patients.