Anti-FolRα ADC STRO-002 Induces Immunogenic Cell Death (ICD) to Enhance Anti-Tumor Activity

Abstract #5591

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Introduction

- STRO-002 is anti-FolRα targeting ADC currently in Phase I clinical trials for the treatment of patients with ovarian and endometrial carcinomas.
- Sutro's proprietary Xpress Cell-Free (XpressCF+TM) technology allows site specific conjugation of the novel cleavable 3-aminophenyl hemiasterlin drug-linker SC239 to generate the specific, homogeneous STRO-002 ADC.
- We previously showed that STRO-002 has immunogenic cell death (ICD) properties and potentiates PD-L1 blockade.
- Here we provide a direct link between *in vitro* ICD and *in vivo* activity, as well as further explore the potential advantages of complementary immune-modulatory activity of STRO-002 on efficacy.

STRO-002 is an anti-FolRα ADC with potent, target-dependent efficacy

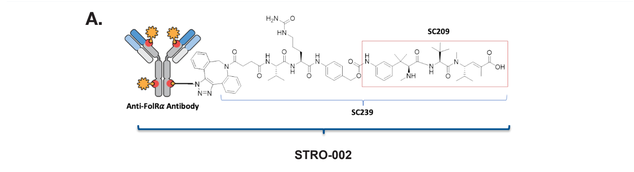


Figure 1 A. STRO-002 consists of a high affinity anti-FolRα antibody conjugated to a novel cleavable 3-aminophenyl hemiasterlin linker-warhead (SC239) at a DAR of 4. The released cytotoxic warhead SC209 is indicated in the red box. **B.** STRO-002 showed potent cell killing activity in FolRα-positive Igrov-1 cells, while control test articles including unconjugated anti-FolRα antibody and anti-GFP antibody conjugated to SC239 (DAR4) were inactive. **C.** A single dose of 10 mg/kg STRO-002 induced tumor regression in Igrov-1 tumors. Equivalent doses of control test articles had no activity. **D.** Dose dependent STRO-002 efficacy is observed in Igrov-1 tumors starting at 2.5 mg/kg.

STRO-002 induces hallmarks of immunogenic cell death (ICD) in FolRα-expressing tumor cells

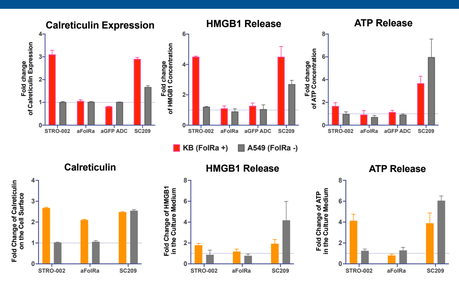


Figure 2. STRO-002-induced translocation of calreticulin on the cell surface, as well as release of HMGB1 and ATP into the cell culture medium on FolRα-positive cells KB and MC38-hFolRα. The active metabolite of STRO-002, SC209, induced ICD markers on both FolRα-positive and negative cells. Unconjugated anti-FolRα antibody and an anti-GFP-SC239 conjugate were used as negative controls.

STRO-002 activates monocytes when co-cultured with FolRα-expressing tumor cells

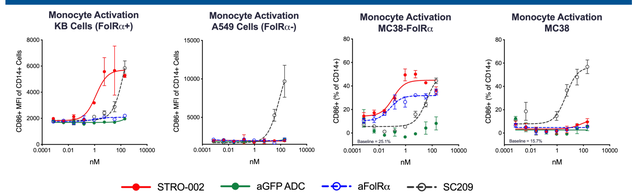


Figure 3. STRO-002 induced ICD resulting in monocyte activation when FolRα-positive cells were cocultured with human PBMCs. SC209 activated monocytes in all cell lines irrespective of FolRα expression. An unconjugated anti-FolRα antibody and an anti-GFP-SC239 ADC were used as negative controls.

In vivo vaccination with STRO-002- and SC209-treated MC38-hFolRα cells confers anti-tumor immunity

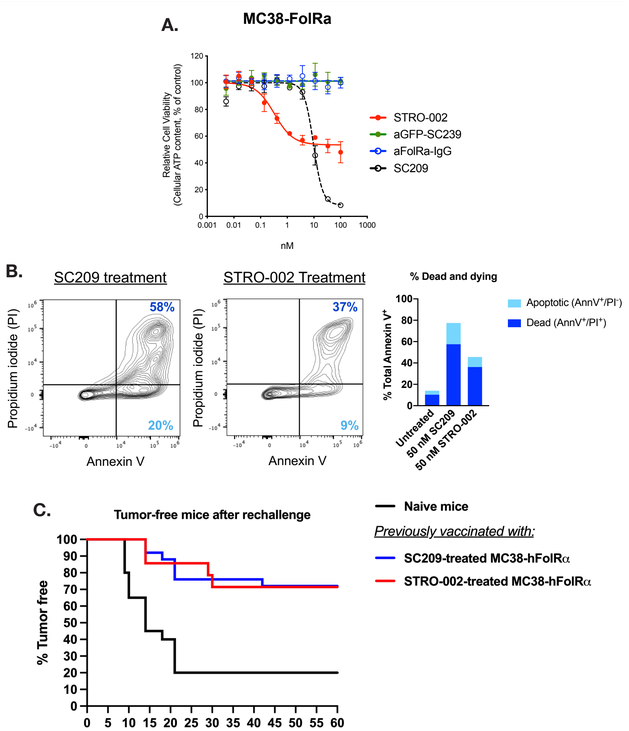
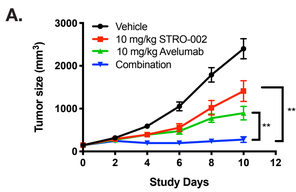


Figure 4 A. Murine MC38 cells engineered to express hFolRα (MC38-hFolRα) treated *in vitro* for 72 hours with STRO-002 and SC209 achieved 50% and 100% cell death, respectively. **B.** Contour plots and bar graphs of annexin V (AnnV) and propidium iodide (PI) staining of vaccine inoculum. MC38-hFolRα cells treated with SC209 or STRO-002 showing 78% and 46% dead-and-dying cells, respectively. **C.** Kaplan-Meier curves of tumor-free mice following rechallenge in immunocompetent C57BL/6 mice showing vaccination with SC209- and STRO-002-treated MC38-hFolRα cells prevented tumor growth in 72% and 71% of animals, respectively, compared to 20% tumor-free mice among naïve animals.

STRO-002 synergizes with Avelumab resulting in durable anti-tumor immunity with evidence of epitope spreading



Treatment	TGI (Day 10)	Complete Responders
STRO-002	44%	0/10
Avelumab	67%	0/10
Combination	**94%**	**14/35 (40%)**

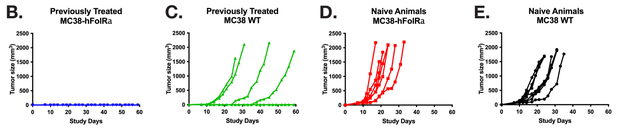


Figure 5 A. Single dose STRO-002 in combination with Avelumab (anti-PD-L1, q3dx2) resulted in significant MC38-hFolRα tumor growth inhibition compared to either single agent. Combination treatment also induced 40% complete regression (CR), while no CRs were observed in other groups. Complete responders were rechallenged with MC38-hFolRα or MC38 WT cells. **B-C.** No tumor recurrence in previously treated animals rechallenged with MC38-hFolRα, while 43% (3/7) remained tumor-free when inoculated with MC38 WT. **D-E.** As expected, all control naïve animals rechallenged with either cell line rapidly developed tumors.

STRO-002 in combination with VEGF blockade exhibits added benefit compared to monotherapy in human OV-90 tumors

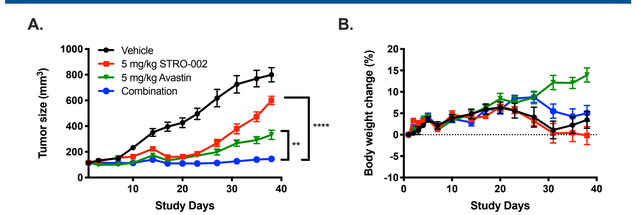
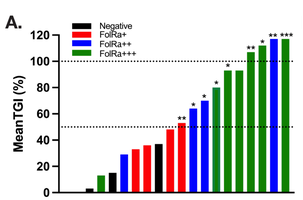


Figure 6 A. Co-administration of STRO-002 and Avastin (anti-VEGF), both administered once, significantly improved tumor growth inhibition (96% TGI) on day 38 compared to STRO-002 (20% TGI) or Avastin (68% TGI) alone in SCID mice bearing human OV-90 tumors. **B.** Percent body weight change calculated relative to animal weight at the start of study shows all treatments were well tolerated.

STRO-002 demonstrates significant activity in human endometrial PDX models with a range of FolRα expression



FolRa Expression	Significant % TGI	Incidence of Response
Negative	0/3	0%
FolRα +	1/4	25%
FolRα ++	3/6	50%
FolRα +++	5/7	71%
Total FolRα Positive	**9/17**	**53%**

Figure 7 A. STRO-002 was significantly efficacious in 53% (9/17) of the FolRα-positive human PDX models in athymic nude mice. The bar graph shows mean TGI for 20 endometrial PDX in response to STRO-002 treatment (qwx4). Models are color coded based on FolRα expression levels. Lines indicate 50% and 100% TGI, while asterisks denote statistical significance. **B.** Table summarizing statistical analysis of TGI using an unpaired t test and incidence of response for PDX models grouped by expression level. High FolRα models show highest incidence of response, while some low and medium FolRα models also exhibit significant activity.

Single dose of STRO-002 induces tumor regression and significant efficacy in ahuman NSCLC PDX model

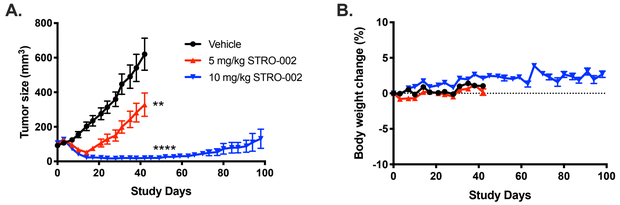


Figure 8 A. Both STRO-002 dose levels initially induced NSCLC PDX tumor regression and demonstrated significant anti-tumor activity compared to vehicle control in athymic nude mice. Continued monitoring of 10 mg/kg STRO-002 group showed prolonged tumor growth suppression for up to three months. **B.** Percent body weight change calculated relative to animal weight at the start of study shows STRO-002 was well tolerated.

Conclusions

- STRO-002 is a bona fide ICD inducer that can elicit host immune system engagement and potentiate efficacy in a target dependent manner.
- Induction of ICD can extend a complementary anti-tumor mechanism in combination therapy regimens with VEGF blockade or checkpoint inhibitors.
- Combination of STRO-002 and Avelumab resulted in complete remissions and durable anti-tumor immunity with evidence of epitope spreading.
- The potent cytotoxic and immunostimulatory properties of STRO-002 may also enhance efficacy in indications with low FolRα expression such as NSCLC and endometrial cancers.

Methods

Cell killing assay: Cells were incubated with indicated test articles at 37°C in CO_2 incubator for 120 hrs. Viability was measured using Cell Titer-Glo® reagent and normalized using untreated cells as control. Data was fitted using GraphPad Prism.

ICD assays: FolRα positive and negative cells were treated for 2 days with indicated test articles. Calreticulin was measured by FACS using a fluorescent labeled anti-calreticulin antibody, HMGB1 measured by ELISA, and ATP release measured by a chemiluminescence based assay. Monocyte activation was indicated by increase of CD86 expression on CD14+ cells in human peripheral blood mononuclear cells (PBMCs) co-cultured with FolRα positive and negative cells.

***In vivo* vaccination:** MC38-hFolRα cells were treated with either 50 nM SC209 or 50 nM STRO-002 for 72 hours. Dead cells in suspension were collected along with adherent cells, detached using enzyme-free dissociation buffer, and resuspended in PBS to form the vaccine inoculum. The vaccine inoculums were assessed for apoptotic cells by using the Annexin V Apoptosis Kit (Biolegend) and were read on an Attune NxT flow cytometer. For vaccination, 1 x 10^6 total cells were injected subcutaneously into the left flank of immunocompetent female C57BL/6 mice. 14 days later, mice were rechallenged at the contralateral flank with 2 x 10^5 untreated MC38-hFolRα cells. As a control, naive mice were also injected subcutaneously with the same cells on the right flank. Tumor take and growth was monitored for 60 days post rechallenge.

***In vivo* efficacy studies:** Igrov1 (SCID Beige), MC38-hFolRa (C57BL/6), and OV-90 (SCID) cells were inoculated subcutaneously into the right hind flank of indicated mouse strain. For endometrial and NSCLC PDX studies, tissue fragments were implanted into athymic nude mice. Treatment with indicated test articles were initiated when tumors were established (150 mm^3) and administered intravenously. Clinical grade Avelumab or Avastin was used for in vivo studies. Most data are presented as mean values ± SEM. Statistical analysis on tumor size at indicated day was performed in Prism using one-way ANOVA with Dunnett's or Tukey's multiple comparisons test, unless otherwise indicated. A probability of less than 5% ($p<0.05$) was considered as significant. Legend: ****, $p<.0.0001$; ***, $p<0.001$; **, $p<0.01$.

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