**Supplemental Figures and Tables for:**

**Discovery of STRO-002, a Novel Homogeneous ADC Targeting Folate Receptor Alpha, for the Treatment of Ovarian and Endometrial Cancers**

**Authors and affiliations**

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**Supplemental Materials and Methods**

**FACS Based FolR𝛼 Receptor Copy Number**

The receptor copy number on the cell surface was determined using Quantum™ Simply Cellular® anti-human IgG beads from Bangs Laboratories (Fishers, IN) according to the manufacturer’s description. Briefly, cells were harvested using Accutase® and counted by the Vi-CELL Cell Viability Analyzers. The Bangs beads were washed separately and re-suspended in FACS buffer (PBS buffer supplemented with 1% bovine serum albumin, 0.05% sodium azide). A total of 200,000 cells or beads per well were incubated with 50 nanomolar (nM) SP8166 conjugated to Alexa647 on ice for 60 minutes. The beads and cells were then washed twice with ice-cold FACS buffer and analyzed in parallel using the BD FACS Canto system. Geometric MFI of each population of the beads was used to plot a linear standard (MFI vs ABC) using QuickCal software from Bangs Laboratories. ABC (Antibody Binding Capacity) of each cell line was interpolated from the standard curve based on the geometric MFI of the cells at the same concentration. The detection limit of the ABC assay is calculated using QuickCal software based on the binding MFI of labeled antibody on the blank beads.

**Cell Cycle Analysis**

FolRα positive KB cells were seeded at 2.5 × 105 cells per well on a 12-well plate overnight. The cell culture medium was replaced with fresh medium the next day and cells were then treated with different concentrations of ADC or free drugs for 48 hours. At the end of treatment time, cells were disassociated with Accutase (VWR) and washed with PBS. The cells were then collected and fixed by adding 1 mL of cold 66% ethanol in PBS for 2 hours at 4°C. After two washes in PBS, cells were treated with 300 µl of 50 µg/ml propidium iodide (Sigma) and 100 µg/ml of RNase (Qiagen) in PBS for 30 min at 37°C in a CO2 incubator. The DNA content of the cells were read by a FACS Canto. The percentage of cells at each cell cycle stage were analyzed using Flowjo software.

**Table S1 Expression of FolR𝛼 in Different cell lines**

|  |  |
| --- | --- |
| **Cell Line** | **FolR𝛼 Receptor Copy Number** |
| KB | 4,107,600 |
| Igrov1 | 1,375,800 |
| OVSAHO | 842,700 |
| OVCAR3 | 196,400 |
| OV90 | 97,700 |
| A549 | 35,100 |

**Table S2 FolR𝛼 expression on patient tumor samples determined by IHC**

|  |  |  |  |
| --- | --- | --- | --- |
| Tumor Sample | | Ovarian Cancer | Endometrial Cancer |
| Total Number of Cores | | 90 | 97 |
| Frequency Score | 0 | 10.00% | 7.20% |
| 1 | 3.30% | 13.40% |
| 2 | 7.80% | 17.50% |
| 3 | 14.40% | 30.90% |
| 4 | 64.40% | 30.90% |
| Intensity Score | 0 | 10.00% | 7.20% |
| 1 | 10.00% | 15.50% |
| 2 | 15.60% | 23.70% |
| 3 | 64.40% | 53.60% |



***Figure S1. In vitro cell-based cytotoxicity assays to test different variants of anti-FolRa ADCs generated by Sutro’s XpressCF+TM technology. A****. Cytotoxicity of different FolRa antibodies conjugated to drug linker SC239 at DAR=4 on the same conjugation sites.* ***B****. Cytotoxicity of ADC variants with different DAR (2, 4 or 6) generated on the same anti-FolRa antibody and conjugated to the same linker-warhead SC239.* ***C****. Cytotoxicity of DAR=4 ADC variants conjugated to SC239 at different conjugation sites on the same FolRa antibody.* ***D****. Cytotoxicity of DAR=4 ADC variants with two different anti-FolRa antibodies conjugated to two different cleavable linkers with the same payload SC209.*



***Figure S2. In vivo assessment in FolRa positive models differentiated and identified optimal ADC parameters.*** *To identify the optimal ADC components, a series of efficacy studies each focused to evaluate a single ADC component while keeping all other parameters constant. In all studies, established KB or Igrov1 tumors were treated with a single dose of FolRa ADC variants at indicated dose level. A. Efficacy of ADC variants with different FolRa antibodies conjugated to the SC239 drug linker at Y180/F404 sites in KB tumors. B. Efficacy of DAR=4 ADC variants with the same FolRa H01 antibody conjugated to SC239 at different conjugation sites in KB tumors. C. Efficacy of DAR=4 ADC variants with two top anti-FolRa antibodies (from Fig. S2A) conjugated to SC239 at two conjugation site combinations (from Fig. S2B) in Igrov1 tumors. D. Efficacy of DAR=4 ADC variants with two different cleavable linkers conjugated to best antibody and conjugation site combination (from Fig. S2C). The H01-Y180/F404-SC239 ADC was selected as optimized lead with the best efficacy, and later designated as STRO-002.*



***Figure S3. SC209 and STRO-002 induced cell cycle arrest and apoptosis on FolR𝛼 positive KB cells.*** *KB cells were treated for 48 hours with indicated test articles. DNA analysis showed that SC209 and STRO-002 greatly reduced cells in G0-G1 phase and significantly increased cell population in G2-M phase. The number of cells in the apoptotic phase was also slightly increased after SC209 and STRO-002 treatment. No meaningful changes were observed with the unconjugated anti-FolR𝛼 antibody SP8166 or an isotype control ADC (an anti-GPF antibody conjugated to SC239 at the same sites).*



***Figure S4. P-gp expression on MES-SA and MES-SA/MX2 cells.*** *MES-SA and MES-SA/MX2 cell were stained with a monoclonal mouse anti-human P-gp antibody, followed by detection with a secondary antibody (Alexa 647 labeled donkey anti-mouse Fc antibody). Histograms show P-gP is highly expressed on MES-SA/MX2 cells, but not on MES-SA cells.*



***Figure S5. FolR𝛼 expression detected by a mouse anti-FolR𝛼 antibody in IHC staining of tumor cells, mouse xenograph tumors, and patient tumor samples.*** *A. Representative images of FolR𝛼 expression on**KB, Igrov1, OVCAR3 tumor cell pellets. B. FolR𝛼 expression on**Igrov1, OVCAR3 and A549 xenograph tumors harvested from mice. C. FolR𝛼 expression on**tumors from ovarian cancer patients representing varying levels of FolR𝛼 levels.*