Discovery and Activity of STRO-002, a Novel ADC Targeting Folate Receptor Alpha for Ovarian and Endometrial Cancer

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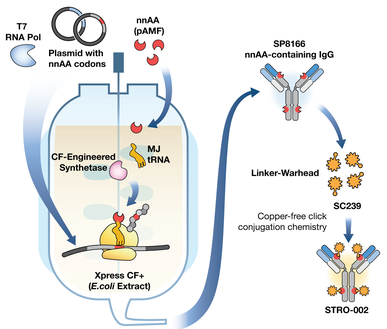
Introduction

- Folate receptor alpha (FolRα) is a cell-surface protein overexpressed in ovarian and endometrial cancer.
- FolRα expression is highly restricted on normal tissues, making it a promising target for cancer therapy using antibody drug conjugates (ADCs).
- We have used a platform based on Sutro's proprietary XpressCF+™ cell-free expression system and site specific conjugation to design a novel, FolRα-targeting ADC, STRO-002.
- STRO-002 contains the anti-FolRα human IgG1 antibody SP8166 (H01) conjugated to a novel proprietary cleavable drug-linker (SC239) at specific sites Y180 and F404 on the antibody heavy chain.
- SP8166 (H01) was discovered and optimized using a Fab ribosome display selection and has four non-natural amino acid, p-azido-phenylalanine (pAMF) residues incorporated at positions Y180 and F404 on each heavy chain.
- SC239 is composed of a tubulin-targeting 3-aminophenyl hemiasterlin warhead, SC209, and a cleavable valine citrulline p-aminobenzyl carbamate linker functionalized with dibenzocyclooctyne (DBCO).
- The rapid and selective reaction of DBCO on SC239 and pAMF residues in SP8166 results in a well-defined, homogeneous ADC with a drug-antibody ratio (DAR) of ~4.

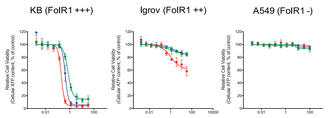
Generation of STRO-002, A Homogenous FolRα-Targeting ADC Through Cell-Free Antibody Synthesis And Site-Specific Conjugation



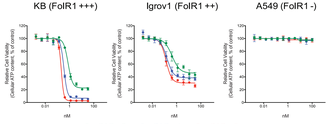
Sutro's proprietary XpressCF+™ cell-free expression system includes:
- incorporation of pAMF, that enables site-specific conjugation,
- an orthogonal tRNA synthetase which enables high fidelity pAMF incorporation,
- engineered XpressCF+™ extracts that utilize an engineered, attenuated RF-1 which enables:
  a. Efficient and multiple insertion points of pAMF in the same translation product
  b. Precise DAR ranging from 1-8 in a single molecular species ADC
  c. ADC production in a few days allowing for rapid iterative structure-activity optimization
- anti-FolRα antibodies isolated using Fab-based ribosome display were optimized for
  - Linker and warhead attributes
  - Antibody moiety
  - Drug antibody ratio
  - Site pairs for drug-linker attachment

Selection of Optimum Drug-Linker and DAR
ADC with a Cleavable Drug-Linker at DAR 4 Showed Best Activity

Non-Cleavable Maytansinoid (SC236)

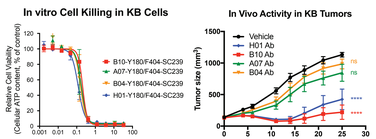


Cleavable 2-Aminophenyl Hemiasterlin (SC239)



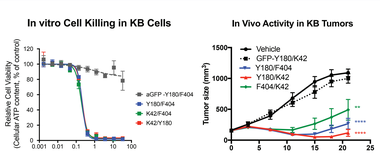
- FolRα ADC variants with different tubulin based drug-linkers, a non-cleavable maytansinoid drug-linker (SC236) or a cleavable 2-aminophenyl hemiasterlin drug-linker (SC239), conjugated to FolRα B10 antibody at DAR = 2, 4, or 6, using the Y180, F404 and/or K42 sites were compared.
- SC239 conjugates exhibited better cytotoxic activity on Igrov1 cells which have lower, but clinically relevant expression levels of FolRα.
- SC239 was therefore selected as the drug-linker for this ADC.
- A conjugate with a DAR of 4 was selected since a DAR of 6 did not provide much higher cytotoxicity than the DAR 4 version.

Selection of Top Antibodies
H01 and B10 FolRα Antibodies Exhibited Best Activity in FolRα Expressing Models



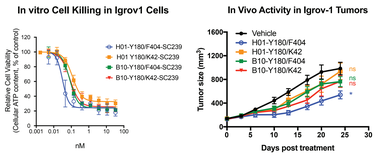
- FolRα ADC variants with different FolRα antibodies (engineered and isolated from Fab ribosome display) conjugated to SC239 at positions Y180 and F404 were compared.
- All variants had comparable *in vitro* cell killing on high expressing FolRα positive KB cells.
- In response to a single 2.5 mg/kg dose, ADC variants with H01 and B10 FolRα antibodies showed significant *in vivo* activity in the KB xenograft model.

Selection of Optimum Conjugation Sites
Conjugation at Sites Y180/F404 or Y180/K42 Showed Best *In Vivo* Activity



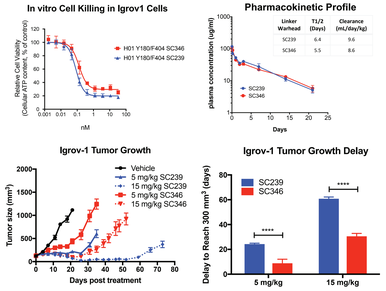
- FolRα ADC variants composed of B10 FolRα antibody conjugated to SC239 at the following site pairs Y180/K42, Y180/F404 or F404/K42 were compared.
- All variants had comparable in vitro cell killing activity on KB cells.
- In response to a single dose at 2.5 mg/kg, ADC variants with SC239 conjugated at Y180/K42 or Y180/F404 demonstrated better *in vivo* activity in the KB xenograft model than the ADC conjugated at F404/K42.

Selection of Final ADC Format
FolRα antibody H01 Conjugated at Y180 and F404 Induced Significant Growth Inhibition of Igrov-1 Tumors



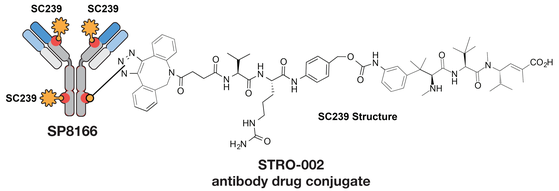
- FolRα ADC variants composed of B10 or H01 FolRα antibodies conjugated to SC239 linker warhead at Y180/K42 or Y180/F404 were compared.
- H01-Y180/F404 showed best *in vitro* cytotoxic activity and *in vivo* tumor growth inhibition in the Igrov-1 model following a single dose at 2.5 mg/kg.

Selection of Optimal Linker
Val-Cit Linker Confered Better Efficacy Than a Val-Ala Linker

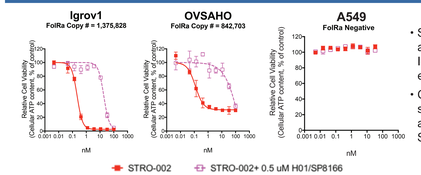


- FolRα ADC variants composed of the H01-Y180/F404 FolRα antibody conjugated to 3-aminophenyl hemiasterlin with either a cleavable Val-Cit drug linker, or a Val-Ala drug linker, were compared.
- Both ADC variants showed comparable pharmacokinetic properties in mice.
- The SC239 conjugate showed slightly improved in vitro cell killing activity and exhibited superior in vivo efficacy than the SC346 conjugate in Igrov-1 model at a single dose of 5 and 15 mg/kg.
- Based on the cumulative data, H01 conjugated to SC239 at positions Y180 and F404 was selected as the lead FolRα targeting ADC, STRO-002..

Structure of STRO-002

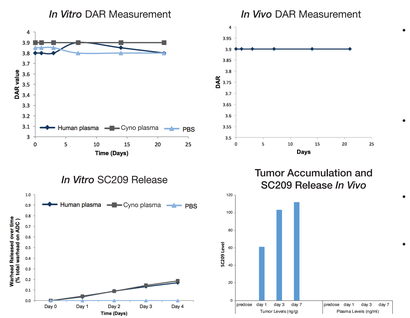


Cell Killing Activity of STRO-002 Is Highly Specific for FolRα Expressing Cells



- STRO-002 showed potent activity in a cell killing assay on FolRα positive Igrov-1 and OVSAHO cells, but no effect on FolRα negative A549 cells.
- Cytotoxic activity of STRO-002 was specific for cells expressing FolRα; and was competed with excess SP8166 (H01).

STRO-002 is Highly Stable *In Vitro* and *In Vivo*



- STRO-002 is highly stable *in vitro* (PBS, cynomolgus or human plasma at 50 μg/mL) and *in vivo* (plasma from mice treated with single dose of 5 mg/kg), with a DAR of ~4 being retained until day 21.
- Minimal release of the STRO-002 catabolite, SC209, was observed after a 4-day incubation of STRO-002 in PBS, cyno or human plasma at 100 μg/mL.
- SC209 was accumulated in tumors, but undetectable in circulation of treated mice bearing Igrov1 tumors.
- STRO-002 was isolated from plasma samples by affinity pull-down and DAR measured by LC-MS. Released SC209 was quantitated by LC-MS.

Summary

- We have leveraged Sutro's XpressCF+™ cell-free technology for rapid interrogation and optimization of parameters for a FolRα targeting ADC, including choice of antibody, conjugation sites, DAR, and linker warhead.
- *In vitro* and *in vivo* assessment showed that FolRα ADC composed of antibody H01 conjugated to SC239 at positions Y180 and F404, designated STRO-002, demonstrated the best anti tumor activity in FolRα expressing models, and was selected as the lead antibody for the program.
- STRO-002 demonstrated good pharmacokinetic and pharmacological properties including specificity, stability and safety in cynomolgus monkeys (not shown here), thus making it an ideal candidate for clinical development.
- IND-enabling studies supporting STRO-002 as a potential treatment of FolRα expressing malignancies are on-going and IND submission is planned for the second half of 2018.

References

- Yin, G., et al.; (2012) Aglycosylated antibodies and antibody fragments produced in a scalable in vitro transcription-translation system. mAbs 4:2, 217-225.
- Zimmerman, E. S., et al.; (2014) Production of site-specific antibody–drug conjugates using optimized non-natural amino acids in a cell-free expression system. Bioconjugate Chemistry 2014, 25, 351–361.
- Yin, G., et. al.; (2017) RF1 attenuation enables efficient non-natural amino acid incorporation for production of homogeneous antibody drug conjugates. Scientific Reports 7(1):3026.