Preclinical safety and efficacy of SOT109, an antibody-drug conjugate targeting Cadherin-17 (CDH17) for the treatment of colorectal and other gastrointestinal tract tumors

Abstract 5469

Nataliia Kalynovska[1], Diana Hudecz[1], Matous Hrdinka[1], Ilona Prochazkova[1], Eliska Kohelova[1], Lenka Palova Jelinkova[1], Filip Jaburek[1], Tomas Zimmermann[1], Lukas Fojtik[1], Irena Adkins[1], Sarka Stehlikova[1], Amy Jensen-Smith[2], Radek Spisek[1], Martin Steegmaier[1]

[1]SOTIO Biotech a.s.; Českomoravská 2532/19b, 190 00 Prague 9, Czech Republic; [2]SOTIO Biotech Inc.; 180 Canal Street, Suite 300, Boston, MA 02114

Abstract

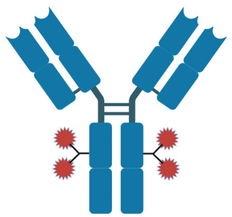
Background: Colorectal cancer is the third most prevalent type of cancer, with an incidence of nearly 2 million new cases annually, and ranks as the second leading cause of cancer mortality worldwide. CDH17 is a single-pass transmembrane glycoprotein involved in calcium-dependent cell–cell adhesion and epithelial homeostasis regulation. In normal physiology, CDH17 is predominantly localized on the lateral surfaces of intestinal and pancreatic ductal epithelial cells. However, it is frequently overexpressed and aberrantly localized in gastrointestinal tumors including colorectal, gastric, pancreatic and gastroesophageal carcinomas. We have developed SOT109, a proprietary CDH17-targeting exatecan-based antibody-drug conjugate (ADC) and evaluated its preclinical safety and efficacy.

Results: Immunohistochemical staining of patient tumor and healthy tissue microarrays confirmed CDH17 expression in normal tissues including the colon, stomach and small intestine, with pronounced expression in colorectal, gastric, and gastroesophageal junction tumors, consistent with previously published literature. SOT109 candidates were generated from fully human CDH17 monoclonal antibodies conjugated via a hydrophilic linker to exatecan, a cytotoxic topoisomerase I inhibitor payload. Candidates were selected by rigorous screening for optimal binding epitopes and affinity, together with binding, internalization, and cytotoxicity *in vitro*. SOT109 candidates demonstrated specific binding to CDH17 and selective internalization and killing of target-positive cancer cells. Lead candidate underwent evaluation in several preclinical *in vivo* mouse tumor models, including cell-derived and patient-derived xenografts. Treatment with SOT109 resulted in profound and sustained tumor regressions. The doses tested in these studies were well tolerated in mice, with no dose-limiting toxicities observed. Subsequent studies in non-human primates revealed a favorable pharmacokinetic and safety profile.

Summary: SOT109 targets the overexpressed tumor antigen CDH17 and exhibits potent anti-tumor efficacy coupled with a clean safety profile in preclinical models of colorectal carcinoma. These data suggest that SOT109 holds significant potential as a therapeutic candidate for patients with gastrointestinal malignancies and supports the further clinical development of SOT109.

SOT109: CDH17-targeting ADC based on the Synaffix technology

Proprietary antibody:
- Fully human mAb
- IgG1 backbone
- Fc domain modification to reduce the effector functions
- Specificity for CDH17 with K_D of 0.15 nM
- No polyspecificity / polyreactivity
- Cross-reactive with mouse, rat and NHP CDH17
- Favorable developability and manufacturability characteristics
- Selected for optimal binding and internalization with respect to target biology



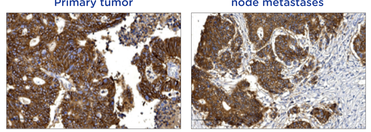
Branched linker:
- Cleavable hydrophilic linker HydraSpace™
- Site-specific and homogenous conjugation to glycan in CH2
- No mAb tags or sequence modifications required for conjugation
- Clinically validated ADC platform

Payload:
- SYNtecan E™
- DAR4
- Payload class and DAR selected for optimal efficacy and safety in relevant indications

Figure 1. Target distribution and prevalence

CDH17 expression in cancer

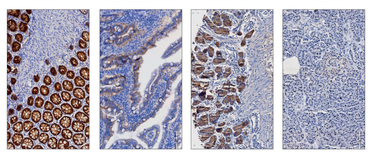
A



Gastrointestinal cancer subtype and tumor location		CDH17 prevalence *(H-score > 75)*
CRC	*primary tumor*	**98%** (n=103)
	metastasis	**91%** (n=44)
Gastric	*primary tumor*	**65%** (n=40)
	metastasis	**58%** (n=81)
GEJ	*primary tumor*	**46%** (n=83)
Pancreatic	*primary tumor*	**11%** (n=36)
	metastasis	**21%** (n=33)

CDH17 expression in normal tissues

B



Adrenal gland	Liver	Prostate
Bladder	Lung	Salivary Gland
Bone marrow	Lymph node	Skeletal muscle
Breast	Mesothelium	Skin
Cerebellum	Nasopharynx	Small Intestine
Cerebrum	Ovary	Spleen
Cervix	Pancreas	Stomach
Colon	Pericardium	Testis
Endometrium	Peripheral nerve	Thymus
Esophagus	Pituitary	Thyroid
Kidney	Placenta	Tonsil

CDH17 staining
No staining

Figure 1. (A) CDH17 expression in colorectal (CRC), gastric, gastroesophageal (GEJ) and pancreatic cancer was assessed using IHC on TMAs. CDH17 is expressed both in primary tumors and metastatic tissue, prevalence is defined as an H score > 75. **(B)** CDH17 expression was confirmed in the healthy colon and, to a lesser extent, in the small intestine, stomach and pancreas. A broad panel of healthy tissues, including vital organs, showed no CDH17 expression confirming a lineage-specific expression pattern for CDH17.

- CDH17 expression profile was assessed in house and is consistent with previously published data
- CDH17 is expressed in primary tumors and metastasis, in all stages of the disease, and serves as a diagnostic marker for CRC

Figure 2. Tumor specificity and anti-tumor rationale

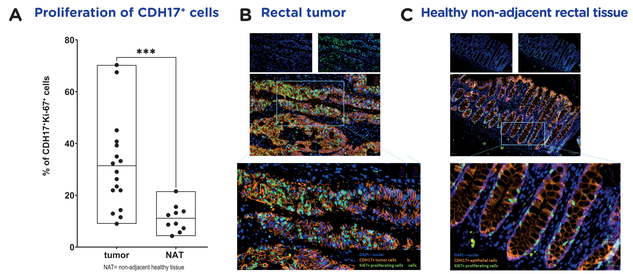


Figure 2. CDH17-positive tumor cells show higher level of proliferation (Ki-67) compared to CDH17-positive epithelial cells in healthy rectum. **(A)** Percentage of CDH17+ Ki-67+ cells in rectal tumor and non-adjacent healthy tissue (NAT) *(N=17 and 10, respectively)*. **(B** and **C)** Graphic representation of co-staining for CDH17 (orange), Ki-67 (green) and DAPI (blue) in rectal tumor **(B)** and healthy tissue **(C)**. In healthy tissue, the expression of CDH17 is present on the lateral surface of enterocytes which show minimal proliferation. In highly proliferative tumor tissue, CDH17 is aberrantly expressed on the entire cell surface.

- Due to the homotypic binding of CDH17 in trans in healthy intestinal tissue, **accessibility** and **internalization** is limited for an ADC, while cancer tissue exhibits disorganized structures resulting in a substantially higher binding and internalization of an ADC
- Unlike healthy enterocytes, target-positive tumor cells exhibit **higher proliferation**, making them more susceptible to the cytotoxic activity of the payload

Figure 3. SOT109 binding to CDH17

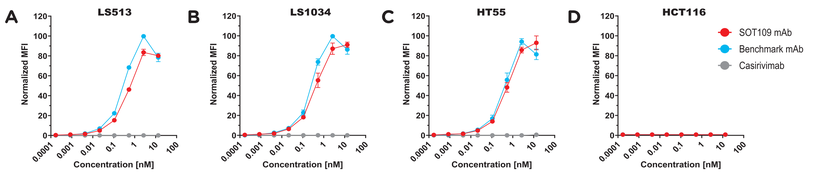


Figure 3. SOT109 mAb binding to high-CDH17- expressing **(A)** LS513, **(B)** LS1034 and **(C)** HT55 and CDH17-negative **(D)** HCT116 cell lines. *Values are presented as normalized mean fluorescence intensity (MFI) to max value, mean+/-SEM, N=3.*

- SOT109 mAb shows target-specific binding to CRC cell lines
- Mean binding EC_{50} value for SOT109 mAb (0.46 nM) is comparable to the clinical benchmark value (0.42 nM)

Figure 4. SOT109 internalization

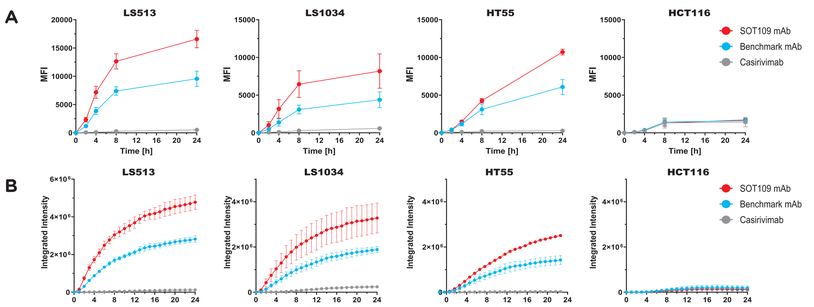


Figure 4. SOT109 mAb internalization in CRC cell lines was followed for 24 hours using two approaches. **(A)** Flow cytometry data. *Values are presented as mean +/-SEM, N=3.* **(B)** Incucyte data. *Values are presented as an antibody integrated intensity per image / cells area confluence (OCU x µm²/Image / %) – normalized to max value (=100%); mean+/-SEM, N=3.*

- SOT109 mAb shows better and faster internalization compared to clinical benchmark
- IC_{50} values for SOT109 cytotoxicity are in sub-nanomolar range for CRC and pancreatic cancer cell lines; benchmark comparably active on pancreatic cancer cell line but only marginally active on CRC cell lines (data not shown)

Figure 5. SOT109 *in vivo* efficacy

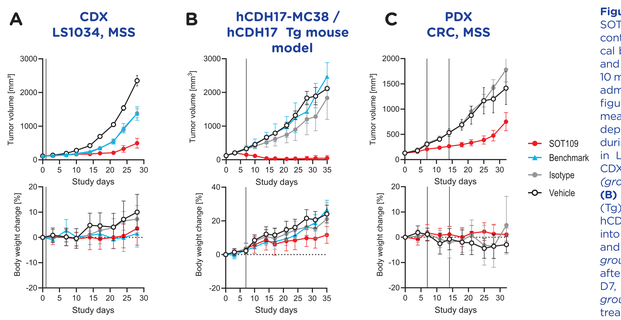
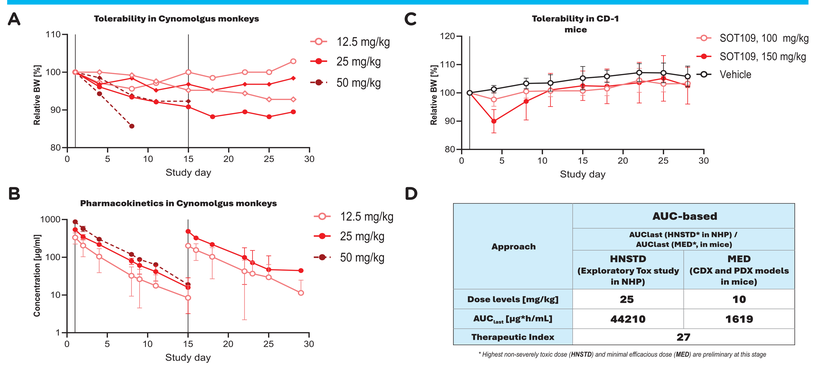


Figure 5. *In vivo* efficacy testing of SOT109 (SYNtecan E™, DAR4), isotype control (SYNtecan E™, DAR4) and clinical benchmark (MMAE, DAR4). SOT109 and isotype control were administered at 10 mg/kg, i.v. and clinical benchmark was administered at 4 mg/kg, i.v. Upper figures refer to the tumor volumes measured over ~ 30 days; bottom panel depicts body weight change observed during the treatment period. **(A)** Efficacy in LS1034 microsatellite stable (MSS) CDX model after a single dose treatment *(group mean+/-SEM, N=5 per group)*. **(B)** Efficacy in hCDH17 transgenic mice (Tg); MC38 tumor cells expressing hCDH17 (hCDH17-MC38) were engrafted into Tg mice. Animals were treated on D0 and D7 *(group mean+/-SEM, N=7 per group)*. **(C)** Efficacy in CRC PDX model after the repeated-dose treatment (D0, D7, D14), *group mean+/-SEM, N=6 per group*. Black vertical line denotes treatment occasion.

- SOT109 demonstrates target-specific efficacy with good tolerability in different models of CRC
- Clinical benchmark conjugated with MMAE shows either limited efficacy or is inactive in some models

Figure 6. SOT109 Pharmacokinetics and tolerability



Approach	AUC-based	
	AUClast (HNSTD* in NHP) / AUClast (MED*, in mice)	
	HNSTD (Exploratory Tox study in NHP)	MED (CDX and PDX models in mice)
Dose levels [mg/kg]	25	10
AUC_{last} [µg*h/mL]	44210	1619
Therapeutic Index	27	

** Highest non-severely toxic dose (**HNSTD**) and minimal efficacious dose (**MED**) are preliminary at this stage*

Figure 6. (A) Tolerability of SOT109 at dose levels 12.5 – 50 mg/kg was assessed in female Cynomolgus monkeys *(N=2 per group)*. SOT109 was dosed via i.v. infusion on D1 and D15. *Individual body weight data (percentage of D1 value) are presented.* **(B)** Pharmacokinetic profiles of SOT109 after the repeated dosing. *Data are presented as concentration of total Ab in plasma, group means+/-SD, N=2.* **(C)** SOT109 tolerability after a single-dose treatment at dose levels up to 150 mg/kg was assessed in female CD-1 mice. *Relative body weight values (% of D1 value) are presented as group mean+/-SD, N=4 per group.* **(D)** Preliminary therapeutic index calculation using AUC-based approach. Exposure levels (AUC_{last}) in CD-1 mice at 10 mg/kg dosing (tentative minimal efficacious does, MED) and in Cynomolgus monkeys at 25 mg/kg (tentative highest non-severely toxic dose, HNSTD) were used for calculations.

- SOT109 was well tolerated in Cynomolgus monkeys and mice at the dose levels up to 25 mg/kg and 150 mg/kg, respectively
- At tolerated doses, no signs of target-mediated toxicity were observed neither clinically (body weight, clinical signs, hematology and clinical biochemistry) nor at microscopical level (histopathology)
- Pharmacokinetic profile observed in Cynomolgus monkeys is sufficient to support Q2W or Q3W dosing in clinical setting
- Preliminary therapeutic index favorable for SOT109 clinical candidate

Summary

SOT109 demonstrates strong anti-tumor activity, surpassing the clinical benchmark, while also exhibiting favorable pharmacokinetic and tolerability profiles. These data suggest that SOT109 displays best-in-class properties offering a promising therapy for patients with CRC and other gastrointestinal tumors.

For more information please contact Nataliia Kalynovska at kalynovska@sotio.com.