A novel topoisomerase I inhibitor antibody-drug conjugate targeting CEACAM5 has potent anti-tumor activity in colorectal cancer models

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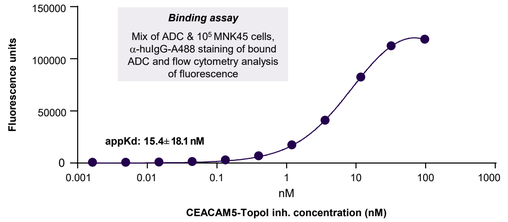
INTRODUCTION

- Carcinoembryonic antigen cell adhesion molecule 5, CEACAM5, is a glycosylphosphatidylinositol-anchored glycoprotein highly expressed on the cell surface of several epithelial tumors[1]
- As determined by IHC, CEACAM5 is expressed at high levels in ~90% of colorectal cancer (CRC)[1]
- Sanofi and Seagen collaboratively developed a novel investigational antibody drug conjugate (ADC), CEACAM5-Topoi inhibitor (Topoi inh), by conjugating a highly selective anti-CEACAM5 antibody from Sanofi with a topoisomerase I inhibitor payload from Seagen that was optimized for potency, reduced Permeability-Glyco-Protein (PGP) efflux and enhanced bystander activity
- In this study we investigated
  - *In vitro* cytotoxicity in cell lines with varying levels of expression of CEACAM5 as well as in vitro cytotoxicity on normal cells not expressing CEACAM5
  - *In vitro* bystander activity
  - *In vivo* efficacy of anti-CEACAM5-Topoi inh. ADC in 4 CRC patient-derived xenografts (PDXs) models
  - *In vivo* efficacy at 10 mg/kg (single administration) in a Single Mouse Trial of 20 CRC PDX models
  - Tolerability in rat after repeated administration (30 or 50 mg/kg/inj., Q1Wx4)

RESULTS

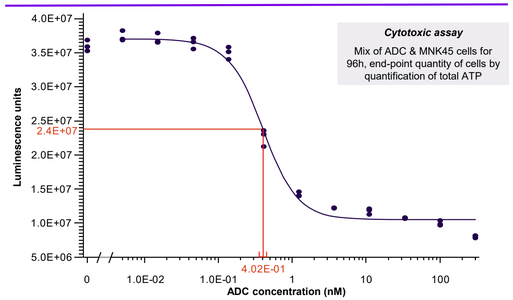
Figure 1: Binding of CEACAM5-Topoi inhibitor ADC to MKN45, cell line with high CEACAM5 antigen density



ADC, antibody drug conjugate; CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; nM, nanomolar; Topoi inh, topoisomerase I inhibitor

- CEACAM5-Topoi inhibitor ADC binds to CEACAM5+ MKN45 cell line with an appKd in the nanomolar range

Figure 2: IC50 for ADC cytotoxicity in MKN45 cells is in the sub-nanomolar range



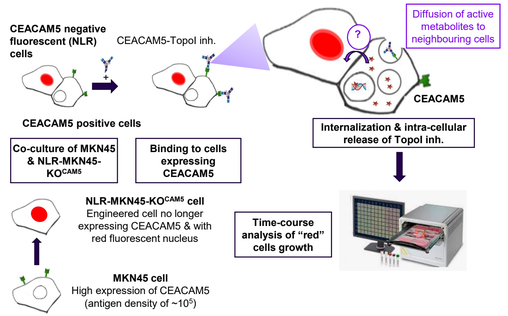
ADC, antibody drug conjugate; ATP, adenosine triphosphate; CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; IC50, half maximal inhibitory concentration; nM, nanomolar

Table 1: IC50 of cytotoxicity for a panel of cells lines with different CEACAM5 antigen densities

	Cell line	CEACAM5 Antigen density	IC50 (nM)
Tumor cell lines	MKN45	500K	0.62 ± 0.19 nM
	LS180	75K	0.40 ± 0.10 nM
	HCT116	0	>300 nM
Normal cell	HUVEC	0	>300 nM
	NHDF	0	>300 nM
	NHBE	0	>300 nM

- **CEACAM5-Topoi inh. ADC IC50 of cytotoxicity is in the sub-nanomolar range for cells lines with high (MKN45) to moderate/low (LS180) CEACAM5 antigen densities**
- **No / very low off-target cytotoxicity on cells not expressing CEACAM5**

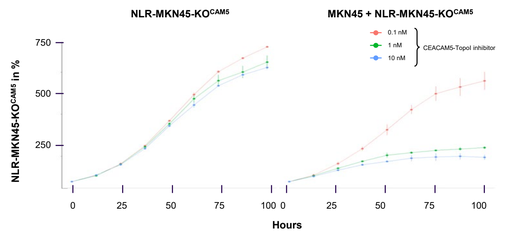
Figure 3: CEACAM5-Topoi inhibitor ADC displays dose-dependant bystander activity



ADC, antibody drug conjugate; CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; KO, knockout; Topoi inh, topoisomerase I inhibitor

Experimental design to analyse bystander effect

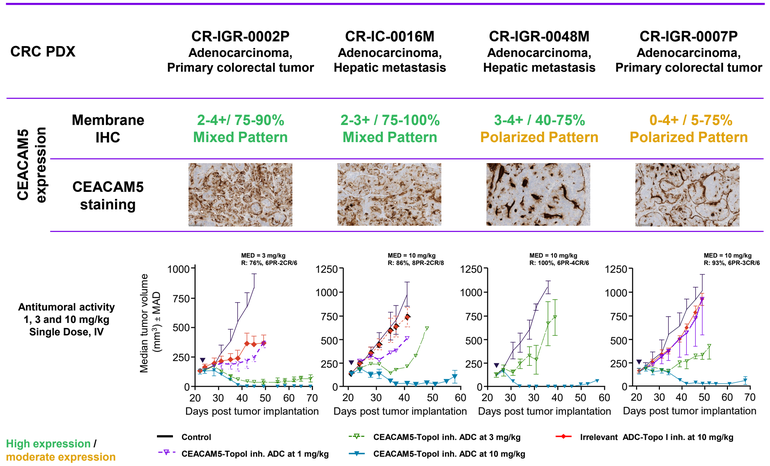
- MNK45 cells were engineered to get cells with fluorescent nucleus & to eliminate CEACAM5 expression, NLR-MKN45-KO^{CAM5}
- Addition of CEACAM5-Topoi inhibitor ADC to NLR-MKN45-KO^{CAM5} or NLR-MKN45-KO^{CAM5} + MKN45
- Time-course analysis of NLR-MKN45-KO^{CAM5} growth



ADC, antibody drug conjugate; CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; KO, knockout; nM, nanomolar; Topoi, topoisomerase I.

- **CEACAM5-Topoi inhibitor ADC induced target-mediated cytotoxicity and bystander effect to neighboring cells, expressing or not CEACAM5**

Figure 4: Robust dose-relationship efficacy of CEACAM5-Topoi inh. ADC in 4 CRC PDX models

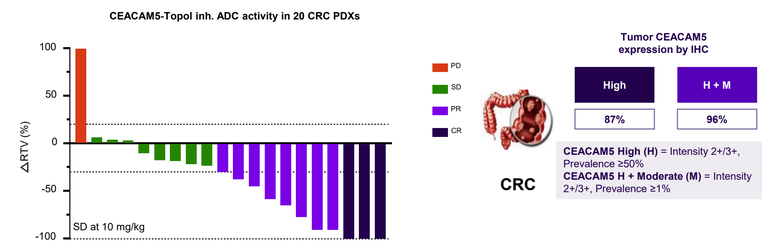


CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; CR, complete regression; CRC, colorectal cancer; IHC, immunohistochemistry; IV, intravenous; MAD, maximum administered dose; MED, minimal active dose; PD, progressive disease; PDX, patient-derived xenograft; PR, partial regression; R, median % of tumor regression ; Topoi inh, topoisomerase I inhibitor; wt, wild type.

- **CEACAM5-Topoi inh. ADC at 10 mg/kg induced robust activity in all 4 PDXs, even in PDX showing moderate and/or heterogeneous CEACAM5 expression.** CEACAM5-Topoi inh. ADC showed dose-dependent activity after a single administration at 1, 3 and 10 mg/kg resulting in tumor regression at 10 mg/kg in all CRC PDX, while an irrelevant ADC was inactive at 10 mg/kg
- SCID mice were implanted subcutaneously with CRC PDX. Single treatment was given as indicated on each figure (▼). Graphs represent the tumor volume evolution by treatment group

Figure 5: Compelling activity of CEACAM5-Topoi inh. ADC in a single mouse trial in CRC preclinical model

- **Principle of the clinical trial in mice:** the Single Mouse Trial (SMT) format employs a single mouse per PDX model and treatment arm across a diverse panel of PDX models, thereby enabling a large-scale, cost-effective in vivo efficacy screen



PDX models are sorted by increasing sensitivity to ADC. The response was determined by comparing tumor volume change at time t to its baseline with ΔRTV = (Vt-V0) / V0 × 100; Criteria for response were adapted from RECIST clinical criteria; Complete Response (CR): Disappearance of tumor; Partial Response (PR):At least a 30% decrease in the tumor volume compared to baseline; Progressive Disease (PD):At least a 20% increase in the tumor volume compared to baseline; Stable Disease (SD):Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

- **CEACAM5-Topoi inh. ADC induced an overall response rate of 55% (including 15 % CR) in CRC PDX models.** The evaluation of CEACAM5-Topoi inh. ADC in SMT shows that displays robust anti-tumor activity in CRC PDX models with high and moderate CEACAM5 expression, supporting further clinical development in monotherapy in this indication

Figure 6: Non-GLP repeated tox study in rat

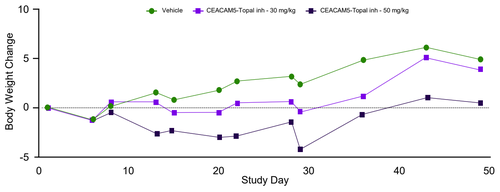
Study design

Group	Test Article	Dose Level[1]	#Animals	Necropsy Timepoints
1	Vehicle	0	6	1 and 4 weeks post last dose (n=3 per timepoint)
2	CEACAM5-Topoi inh	30 mg/kg	6	
3		50 mg/kg	6	

[1]Dose levels were reduced due to hemolysis in n=3 animals in the treated groups on D1. Subsequent evaluation determined hemolysis was driven by the osmolality of the solution

CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; D, day; TK, toxicokinetics; Topoi, topoisomerase I.

Body weight (change from baseline)



CEACAM5, carcinoembryonic antigen cell adhesion molecule 5; Topoi inh, topoisomerase I inhibitor.

- **High dose level caused ~ 5% decrease in body weight up to Day 29**
- **Animals were at or above their baseline weights at the end of recovery period**

Main findings

Findings at end of dosing (Day 29)

- Minor (~5%) reductions in body weights (at high dose)
- Minimal increases in platelets and neutrophils (all groups)
- Moderate to severe lymphoid reductions in the thymus (all groups)
- Minimal increases in alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH)

Findings at end of recovery (Day 50)

- Recovery of body weight, hematology, serum chemistry, and thymus changes

- **CEACAM5-Topoi inhibitor was well tolerated when given to rats weekly for 4 weeks at up to 50 mg/kg**
- **MTD and dose-limiting toxicities were not determined**

CONCLUSIONS

- **CEACAM5-Topoi inh. ADC killed tumor cells with high to moderate CEACAM5 antigen density at sub-nM concentration, while it displayed no/very low cytotoxicity towards CEACAM5-negative cells**
- **Mechanistically, cytotoxicity was mediated by direct killing of CEACAM5+ cells and by bystander effect**
- **CEACAM5-Topoi inh. elicited a potent dose-dependent antitumor activity in 4 CRC PDX models**
- **This robust efficacy was confirmed by an ORR of 55% (including 15% CR) in a CRC PDX Single Mouse Trial**
- **CEACAM5-Topoi inh. was well tolerated when given to rats weekly for 4 weeks at up to 50 mg/kg. MTD and dose-limiting toxicities were not determined**
- **The compelling anti-tumor activity and its favorable safety profile in rats support further evaluation of this investigational novel topoisomerase I ADC in CRC patients**

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1. Decary S, et al. *Clin. Cancer Res*. 2020 Dec 15;26(24):6589-99.

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