

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

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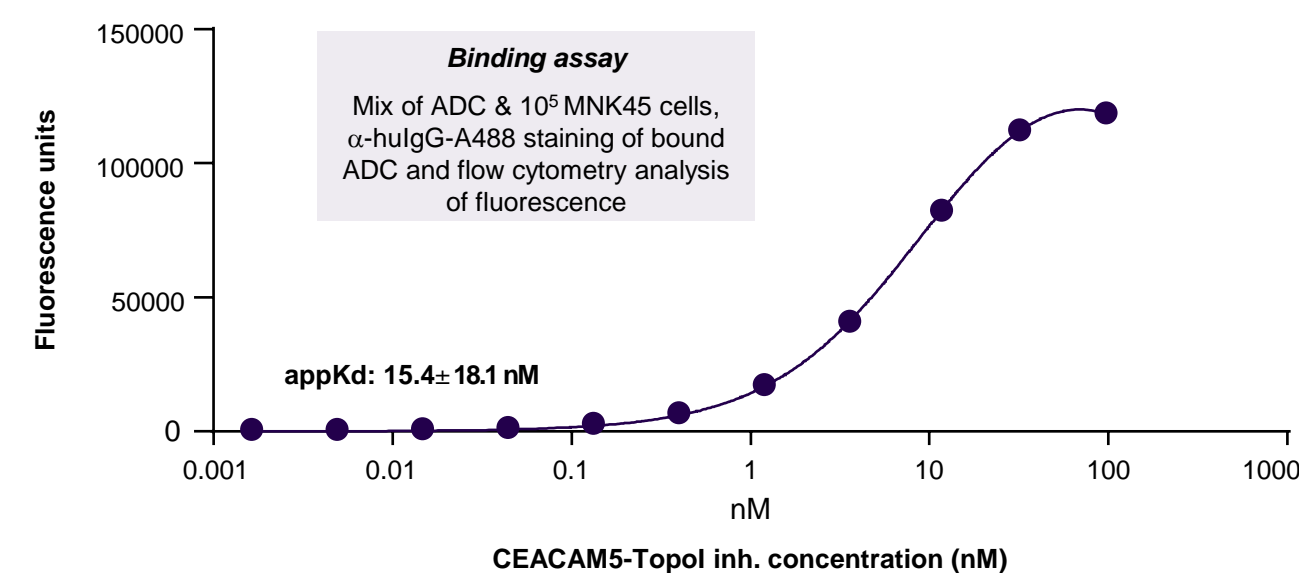
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## INTRODUCTION

- Carcinoembryonic antigen cell adhesion molecule 5, CEACAM5, is a glycosylphosphatidylinositol-anchored glycoprotein highly expressed on the cell surface of several epithelial tumors<sup>1</sup>
- As determined by IHC, CEACAM5 is expressed at high levels in ~90% of colorectal cancer (CRC)<sup>1</sup>
- Sanofi and Seagen collaboratively developed a novel investigational antibody drug conjugate (ADC), CEACAM5-Topol inhibitor (Topol 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-Topol 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

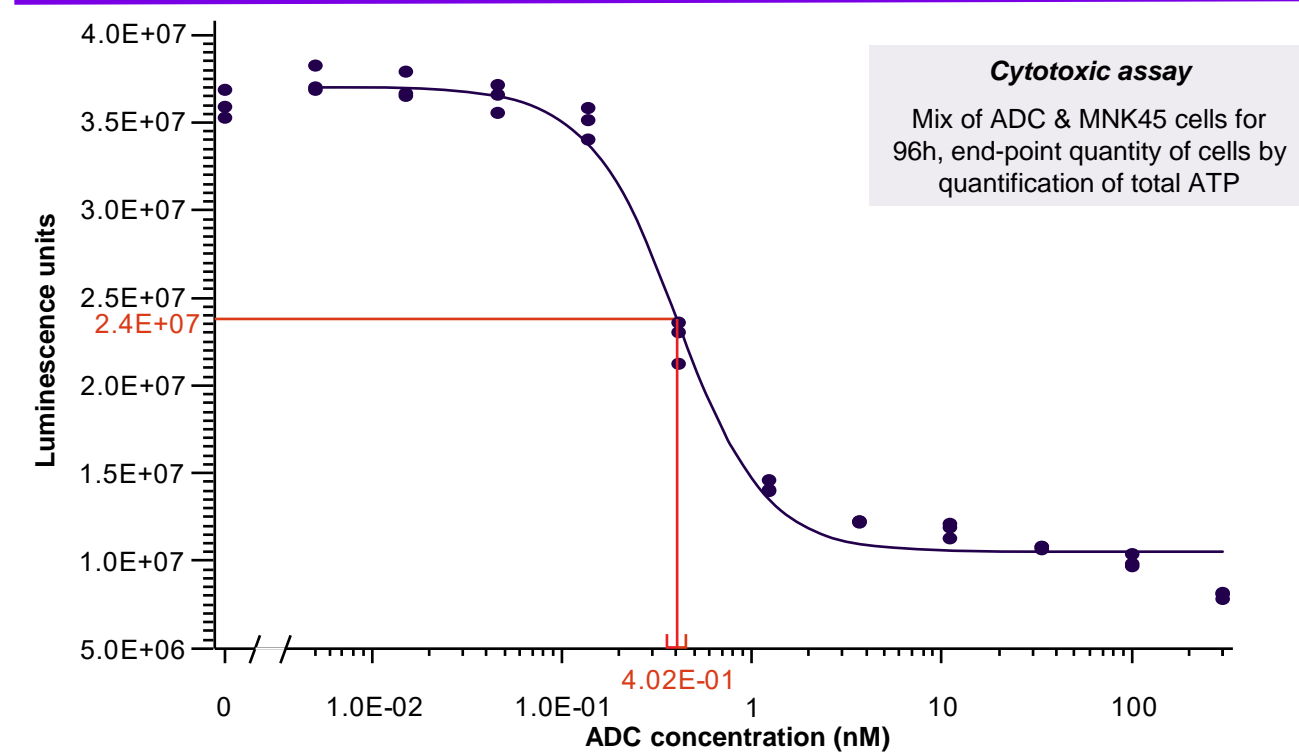
**Figure 1:** Binding of CEACAM5-Topol inhibitor ADC to MKN45, cell line with high CEACAM5 antigen density



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

- CEACAM5-Topol 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

## ACKNOWLEDGMENTS:

All contributors from Sanofi and Seagen

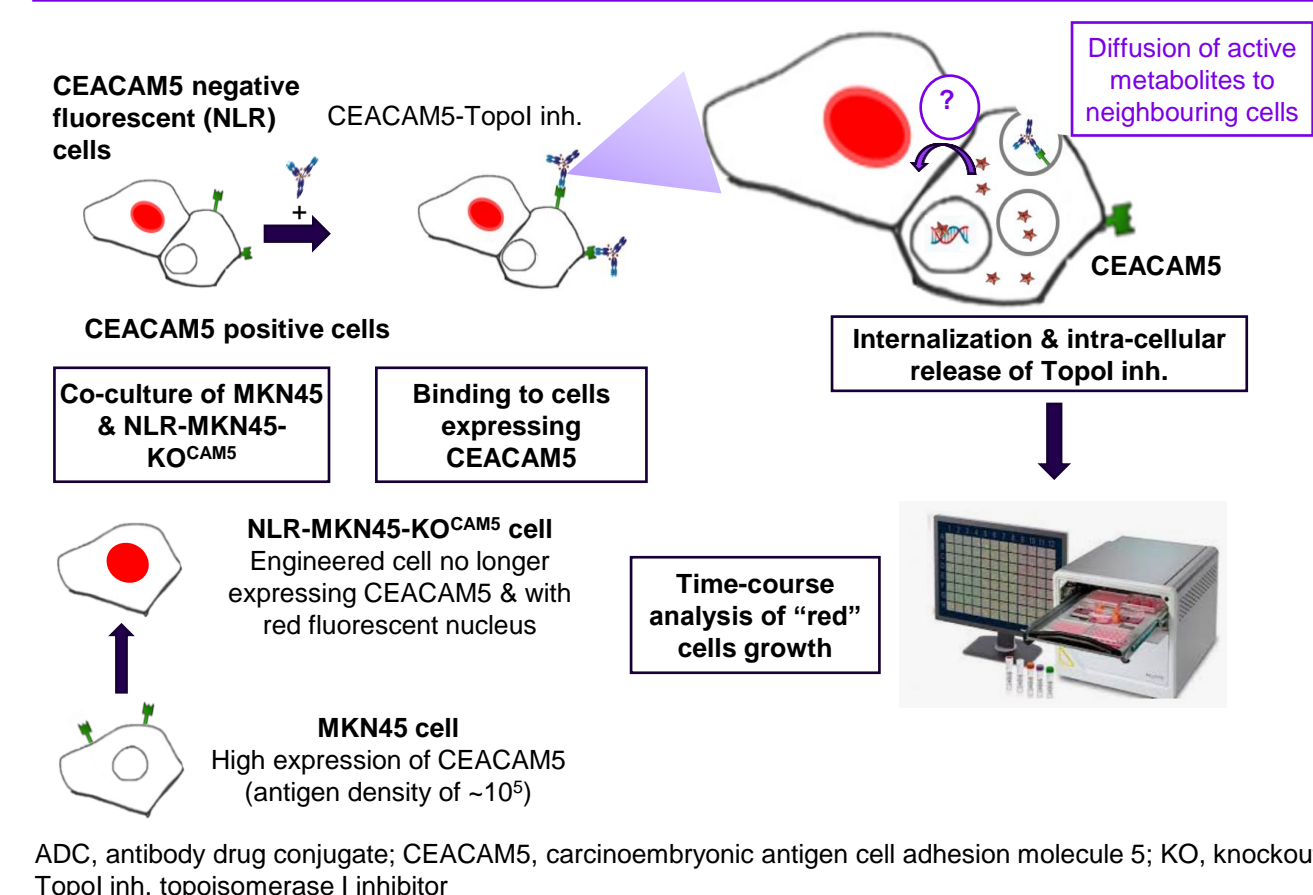
- Carole Jullien who performed binding, *in vitro* cytotoxicity and bystander experiments
- Anne-Marie Lefebvre who did supervised IHC analysis
- Nicolas Moindrot and Ravi Rangara who performed efficacy in PDX models
- Ludvine Coquan-Andrieu as non clinical efficacy and safety statistician expert
- Ajay Francis Christopher of Sanofi provided the editorial support

**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-Topol 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**

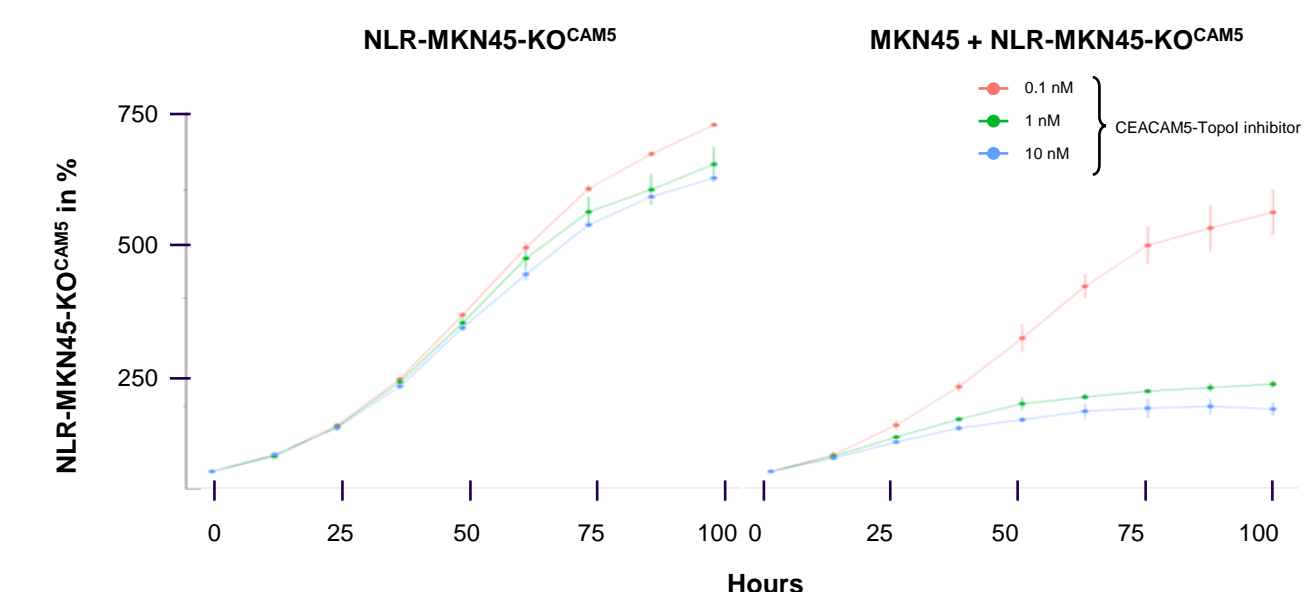
**Figure 3:** CEACAM5-Topol inhibitor ADC displays dose-dependant bystander activity



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

### Experimental design to analyse bystander effect

- MKN45 cells were engineered to get cells with fluorescent nucleus & to eliminate CEACAM5 expression, NLR-MKN45-KO<sup>CEAM5</sup>
- Addition of CEACAM5-Topol inhibitor ADC to NLR-MKN45-KO<sup>CEAM5</sup> or NLR-MKN45-KO<sup>CEAM5</sup> + MKN45
- Time-course analysis of NLR-MKN45-KO<sup>CEAM5</sup> growth



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

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

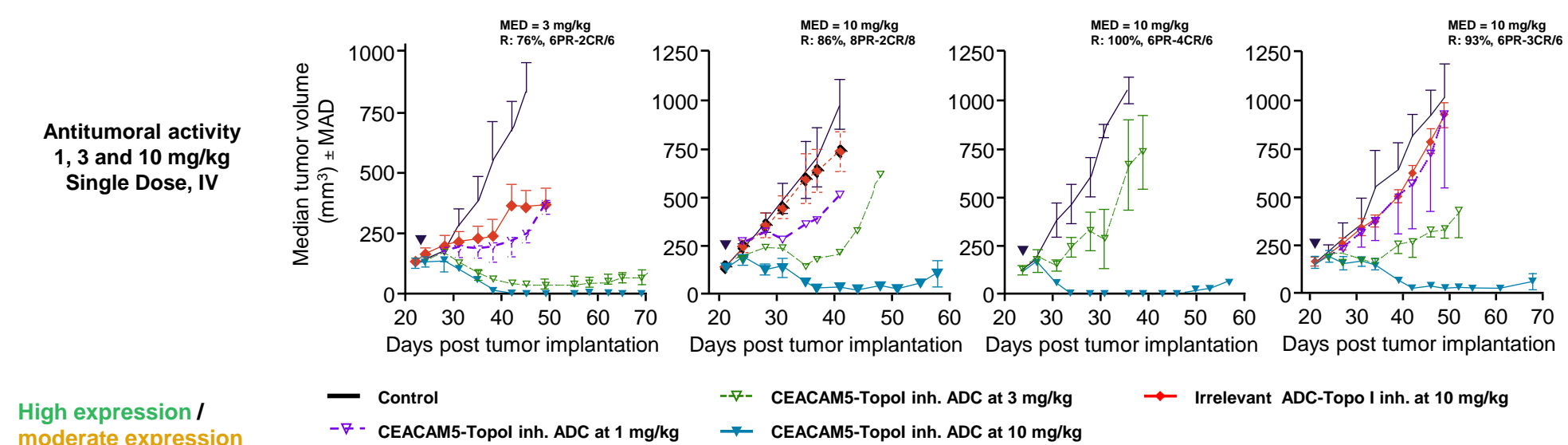
## DISCLOSURES:

Y. Baudat, Sanofi R&D Employment. H. Neff-LaFord, Seagen Employment. C. Nicolazzi, Sanofi R&D Employment. D. Meyer, Seagen Employment. J. Sigurjonsson, Seagen Employment. R. Lyski, seagen Employment. V. Fantin, Sanofi Employment. M. Brun, Sanofi R&D Employment. M. Chiron, Sanofi R&D Employment. S. Decary, Sanofi R&D Employment

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

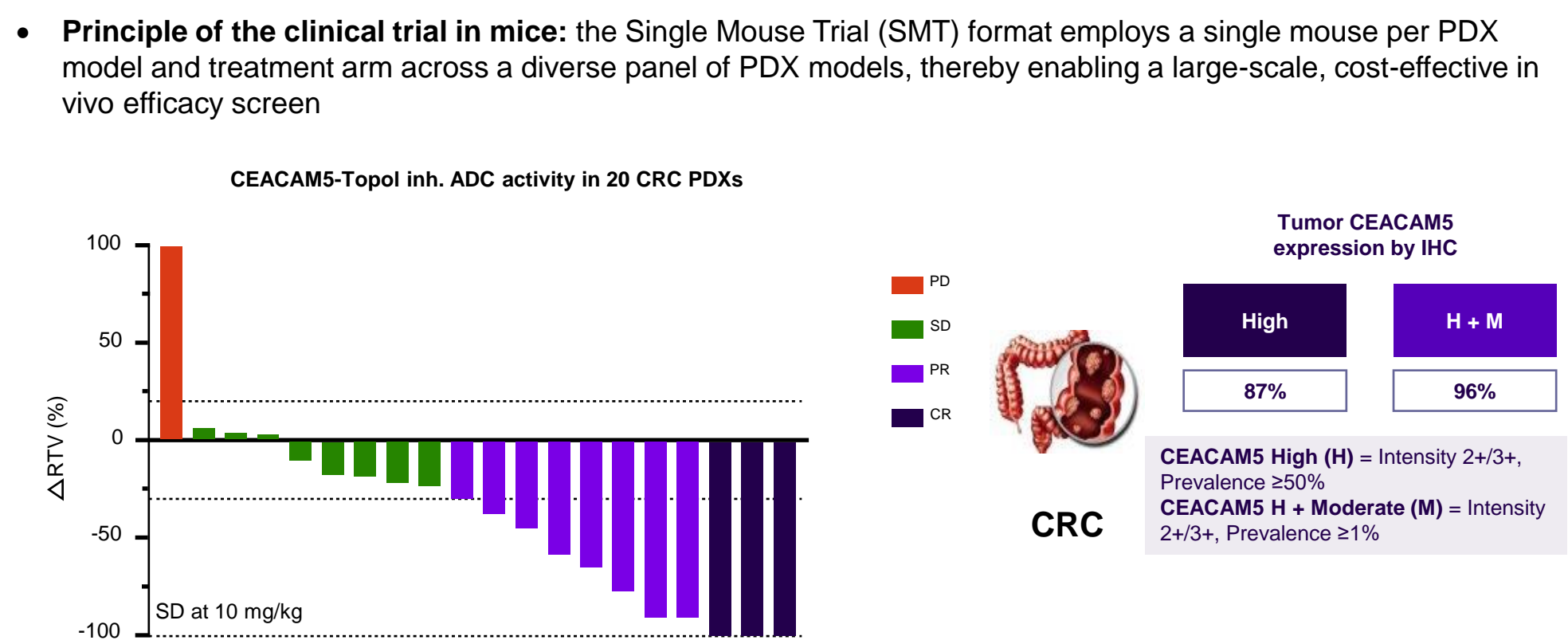
CRC PDX	CR-IGR-0002P Adenocarcinoma, Primary colorectal tumor	CR-IC-0016M Adenocarcinoma, Hepatic metastasis	CR-IGR-0048M Adenocarcinoma, Hepatic metastasis	CR-IGR-0007P Adenocarcinoma, Primary colorectal tumor
CEACAM5 expression	2-4+ / 75-90% Mixed Pattern	2-3+ / 75-100% Mixed Pattern	3-4+ / 40-75% Polarized Pattern	0-4+ / 5-75% Polarized Pattern
Membrane IHC				
CEACAM5 staining				



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; Topol inh, topoisomerase I inhibitor; wt, wild type.

- **CEACAM5-Topol inh. ADC at 10 mg/kg induced robust activity in all 4 PDXs, even in PDX showing moderate and/or heterogeneous CEACAM5 expression.** CEACAM5-Topol 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-Topol inh. ADC in a single mouse trial in CRC preclinical model



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-Topol inh. ADC induced an overall response rate of 55% (including 15 % CR) in CRC PDX models.** The evaluation of CEACAM5-Topol 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

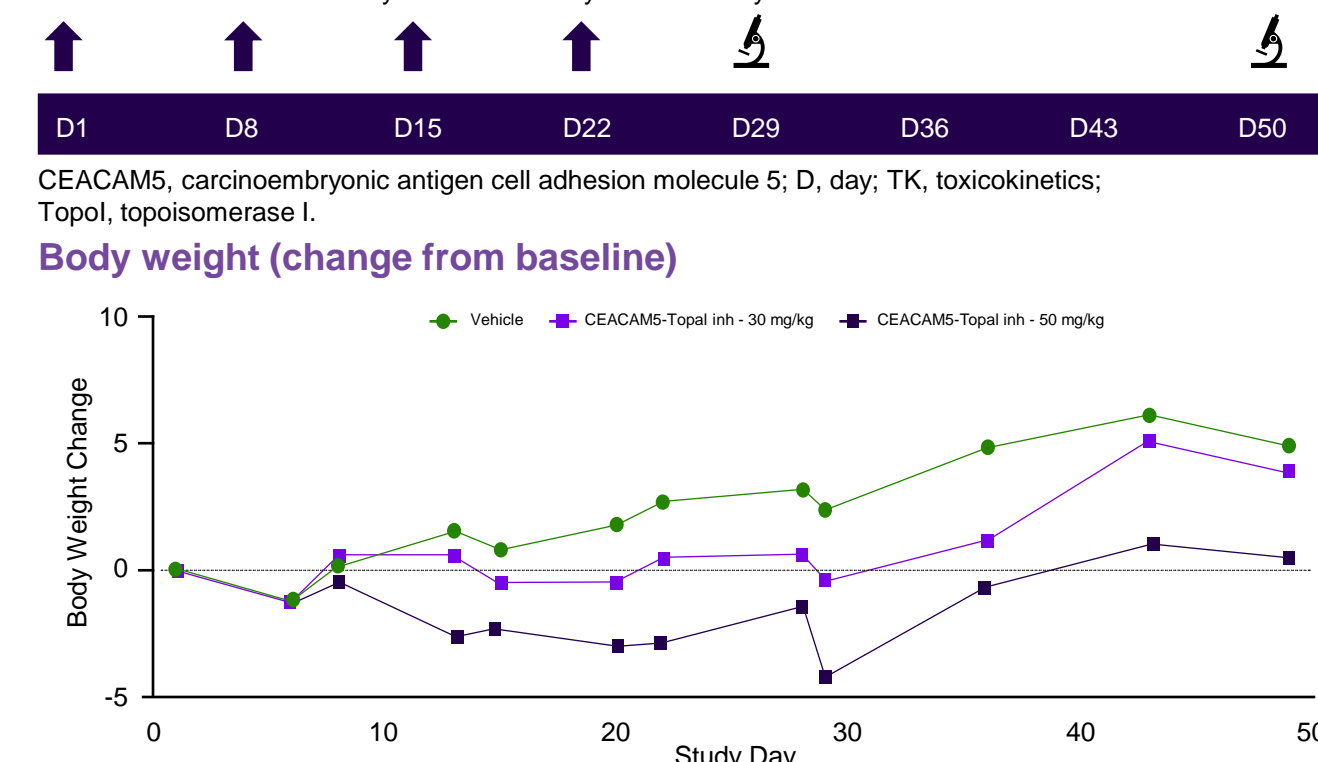
## REFERENCES:

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

**Figure 6:** Non-GLP repeated tox study in rat

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

<sup>1</sup>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; Topol 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-Topol 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-Topol 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-Topol 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-Topol 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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