CUSP06/AMT-707, a Novel CDH6-Targeting Antibody-Drug Conjugate, Demonstrates Potent Antitumor Activity in Preclinical Models

Wei Lu[1], Jing Shi[2], Shu-Hui Liu[2], Nicole Covino[1], Wentao Zhang[1], Xun Meng[2], Eric Slosberg[1]

[1]OnCusp Therapeutics, 433 Broadway, New York, NY 10013, USA
[2]Multitude Therapeutics, Floor 1-3, Building 1, No 333 Guiping Road, Shanghai 200233, China

Abstract Presentation Number: 6320

Abstract

Cadherin-6 (CDH6), also known as K-cadherin, is a type II classic cadherin molecule that plays an important role in the embryonic development of the kidney but has very limited expression in adult tissues. It is overexpressed in several human malignancies, primarily in ovarian, renal, but also (to a lesser degree) cholangiocarcinoma, lung, HCC, Uterine Serous Carcinoma (USC), thyroid cancers and other tumor types. The characteristic of limited expression in normal tissues, high expression in tumor tissues, and rapid internalization upon antibody binding makes CDH6 a well-suited Antibody-drug Conjugate (ADC) target.

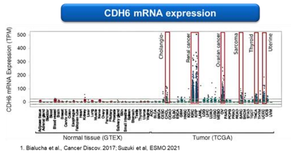
We developed a novel CDH6-targeting ADC, CUSP06, consisting of a proprietary humanized antibody selective for CDH6, a protease cleavable linker, and an exatecan payload, with a drug-to-antibody ratio (DAR) of 8. CUSP06 selectively bound to cell surface CDH6 and was efficiently internalized into CDH6 positive ovarian and renal cancer cells. CUSP06 exhibited strong antiproliferative activity against several CDH6 positive cancer cell lines *in vitro*. Furthermore, compared to DXd-based ADC, exatecan-based ADC demonstrated improved bystander effect. Treatment with CUSP06 resulted in tumor regression in several CDH6 positive cell line derived xenograft (CDX) models, including PA-1, OVCAR3, and 786-O. In addition, CUSP06 demonstrated potent antitumor activity with tumor regression observed in several CDH6 low and high expressing patient derived xenograft (PDX) models.

The preclinical activity of CUSP06 against CDH6-expressing tumor provides compelling support for the clinical development of CUSP06 in CDH6-expressing human cancers. CUSP06 is currently in IND-enabling studies. CUSP06 showed an expected toxicity profile consistent with the exatecan payload in the ongoing pilot toxicology studies. We plan to initiate a Phase 1 first-in-human clinical trial in the 2nd half of 2023.

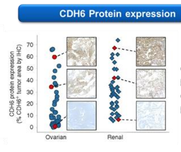
Target Introduction

- CDH6 (K-cadherin) is a type II classic cadherin transmembrane protein, accessible to binding by antibodies, and is then rapidly internalized by endocytic membrane trafficking
- In adults CDH6 is only expressed in kidney, mammary gland and thymus, and at low levels[1]
- High expression of CDH6 is seen in several malignancies including renal cell cancer and ovarian cancer and correlates with tumor progression and poor prognosis[1]
- Elevated expression in subsets of patients with cholangiocarcinoma, lung, HCC, Uterine Serous Carcinoma (USC), thyroid and other cancers
- CDH6 is an attractive antibody-drug conjugate (ADC) target. DS-6000, a CDH6-ADC, is currently in Ph1 trial

CDH6 mRNA expression



CDH6 Protein expression

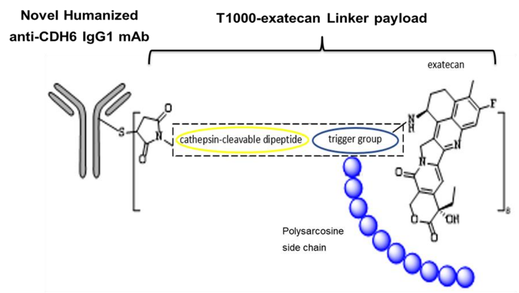


CDH6 positive OvCa patients ≥ 85%
CDH6 positive RCC patients ≥ 95%

1. Baluchа et al., Cancer Discov. 2017; Suzuki et al, ESMO 2021

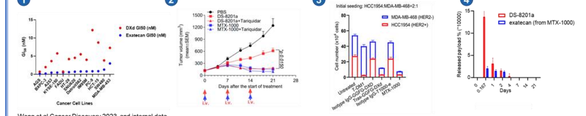
CUSP06 Schema

CUSP06 contains a humanized anti-CDH6 IgG1 monoclonal antibody, which is conjugated to exatecan (a topoisomerase I inhibitor payload) by the proprietary T1000 hydrophilic and enzyme-cleavable linker. CUSP06 has a drug-to-antibody ratio (DAR) of ~8.



CUSP06's T1000-e Linker-Payload has Superior In Vitro Profile to DS-6000's Deruxtecan*

Characteristics		T1000-e (used in CUSP06)	Deruxtecan (used in DS-6000)	Comment
Payload (exatecan)	1 Potency	+++	++	Exatecan (CUSP06) is a more potent TOPO1 inhibitor than DXd (DS-6000)
	2 Overcomes Drug Resistance	+++	+	A much weaker substrate for BCRP and P-gp (common chemoresistance mechanisms) vs DXd
	3 Bystander effect	+++	++	Shows superior bystander effect vs DXd
Linker	4 Stability	++	+	Improved stability of T1000-e vs Deruxtecan in circulation



Weng et al Cancer Discovery 2023, and internal data

* Deruxtecan is the linker-payload used in DS-6000

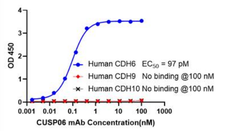
In Vitro Binding Characterization

Both CUSP06 and CUSP06 mAb exhibit high affinity binding for human CDH6 in an ELISA assay, and human CDH6 on OVCAR3 cells via FACS analysis.
CUSP06 mAb exhibits equal binding affinity for human, monkey, rat and mouse CDH6 recombinant protein, and is shown to have >1000-fold selectivity for CDH6 over CDH9 and CDH10, two closely related cadherins.

Binding Affinity (EC_{50}: nM)

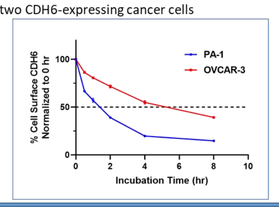
	Human CDH6 (ELISA)	Human CDH6 in OVCAR3 (FACS)
CUSP06 mAb	0.085	0.28
CUSP06	0.072	0.54

CDH6 Binding Selectivity



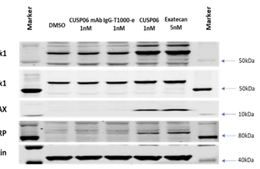
CUSP06 In Vitro Internalization

Proper cellular internalization of an ADC following antigen binding is critical to its tumor-killing function. CUSP06 demonstrates efficient internalization with an average of 50% uptake between 1 and 4 hours in two CDH6-expressing cancer cells



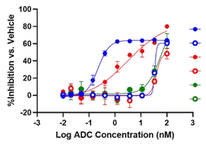
CUSP06 Induces DNA Damage and Apoptosis in OVCAR3 Cells

The ability of CUSP06 to induce apoptosis of tumor cells is shown in the western blots below, where CUSP06 treatment of OVCAR3 cells for 72 hours resulted in increased level of DNA damage biomarkers (pChk1 and pH2AX) and apoptosis biomarker (cleaved PARP). Free exatecan payload also induces apoptosis, while naked CUSP06 mAb or a non-specific ADC (IgG-T1000-e) does not.



CUSP06 Antiproliferation Activity

CUSP06 inhibits ovarian cancer cell proliferation in vitro in the sub-nanomolar to nanomolar range, in a CDH6-dependent manner. The negative control of non-specific ADC (IgG-T1000-e) only kills cells at much higher levels



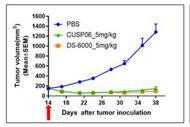
Antiproliferation IC_{50} (nM)

	CUSP06	IgG-T1000-e	CDH6 Expression
OVCAR-3	0.22	73	High
PA-1	3.1	>100	High
ES-2	69	52	Null

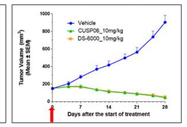
CUSP06 Exhibits Potent In Vivo Efficacy in Three CDH6 Cell Line Derived Xenograft (CDX) Models

The ability of CUSP06 to induce tumor regression in vivo is shown in these 3 CDH6-high expressing ovarian cancer and RCC cell line-derived xenograft models. DS-6000 [DS6000 is made by expressing its mAb in IgG1 backbone based on the CDR sequence from Daiichi Sankyo's patent followed by conjugation with deruxtecan to yield a DAR-8 ADC *include here or put as footnote?] shows similar potency at CUSP06 in the two ovarian models. All ADCs were well tolerated as assessed by body weight.

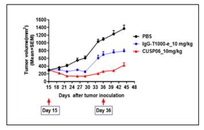
PA-1 (OvCa)



OVCAR-3 (OvCa)

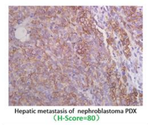


786-O (RCC)

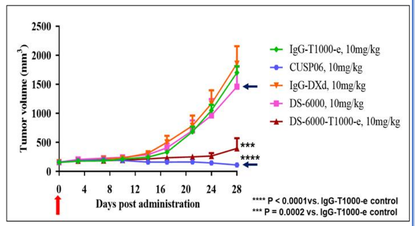


CUSP06 Demonstrates Superior Efficacy Over DS-6000 in a CDH6-Low Kidney Nephroblasma PDX Model

In this CDH6-low expressing kidney cancer (pediatric nephroblastoma / Wilm's tumor) PDX model, DS-6000 has minimal effect on tumor growth, whereas CUSP06 showed potent anti-tumor activity. In order to better understand the driver for this differential effect, we created a chimeric ADC that has the DS-6000 mAb attached to the CUSP06 T1000-e linker-payload. This molecule (DS-6000-T1000-e) had strong activity, confirming that CUSP06's linker-payload drive the majority of the improvement.
One hypothesis for this would be the efflux of DXd due to the Pgp or BCRP drug efflux pumps (which are non-selective for exatecan). Western blots confirmed the lack of Pgp or BCRP protein in this model, thus the improved activity of CUSP06 is most likely due to our payload, which is more potent, higher cell permeability, and has improved bystander effect compared to DXd
CUSP06 also demonstrates activity in another CDH6-low model



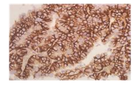
Hepatic metastasis of nephroblastoma PDX (H-Score=80)

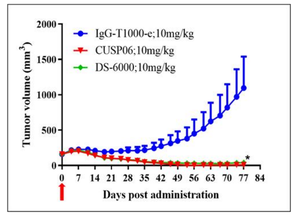


CUSP06 Shows Strong Activity in a PARPi-Resistant Ovarian Cancer Patient Derived Xenograft (PDX) Model

In this clinically relevant, olaparib-resistant ovarian cancer patient derived xenograft (PDX) model, that expresses very high levels of CDH6 (IHC H-Score of 280), a single administration of CUSP06 shows potent, very durable anti-tumor activity. Although DS-6000 shows similar activity, we observe, upon sacrifice at the final time point, that CUSP06 leads to complete loss of tumor in 4 out of 5 mice, compared with only 2 out 5 mice for DS-6000, and 0 out of 6 mice for the IgG-T1000-e negative control ADC. All ADCs were well tolerated as assessed by body weight.

LD-1588 Ovarian cancer PDX
CDH6 H-Score = 280





* P < 0.05 vs. IgG-T1000-e control

CUSP06 Non-Clinical Toxicology Studies

- Cynomolgus monkeys and Sprague Dawley rats were selected as the toxicology species since CUSP06 showed comparable affinity with human, monkey and rat CDH6
- Dose Range Finding (DRF) studies indicate cyno monkey was the more sensitive species to CUSP06
  - In Cyno monkeys, CUSP06 is well tolerated and showed equivalent or potentially better safety profile than DS-6000
  - Primary toxicities were diarrhea and myelosuppression. No renal or lung toxicities were observed
  - In SD rat, CUSP06 is well tolerated at much higher dose
  - CUSP06 exhibited good total antibody and ADC exposure and high linker stability, with very low levels of free exatecan payload, in both monkey and rat studies
- CUSP06 is well-tolerated in the ongoing GLP tox studies in monkeys and rats

CUSP06 Summary and Program Status

- CDH6 is an attractive ADC target
- CUSP06 is a CDH6 targeted ADC consisting of novel hIgG1 mAb conjugated with the proprietary T1000-exatecan linker payload with DAR of 8
- CUSP06 exhibits potent and selective binding to CDH6, undergoes rapid internalization, induces DNA damage and cell apoptosis
- CUSP06 demonstrates CDH6-dependent cell growth inhibition in vitro, and tumor regression in vivo in multiple CDX and PDX models
- CUSP06 demonstrates superior in vivo activity over DS-6000 in CDH6-low PDX models
- CUSP06 is well-tolerated in the ongoing GLP tox studies in rat and cynomolgus monkeys
- CMC work is progressing well and is on track to deliver GMP material in time for Ph1 trial start
- IND filing is projected for 3Q2023 and Phase 1 clinical study start in the 2nd half of 2023

Acknowledgements

The authors acknowledge the contributions from Bing Yuan and Andy Fu for their leadership and strategic input on CUSP06 program. Special thanks to Robert Phillips and John Cogswell (translational medicine), Amy Penticoff (CMC), Rebecca Klouwers, Bryan Zhang and Roy He (figure and poster preparation), and Laurie Tatalick (nonclinical toxicology) for this work. Special thanks to Yanfang Tang, Caiwei Chen, Lijun Wang, Yi Shen, Yixuan Wang and Jianjian Zhang (discovery work for CUSP06/AMT-707) and Chao Kong (project management).