



Preclinical Assessment of HWK-016, a Next-Generation, MUC16-Targeting ADC with Novel Bioconjugation and Linker–Payload Technology



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Disclosure Information



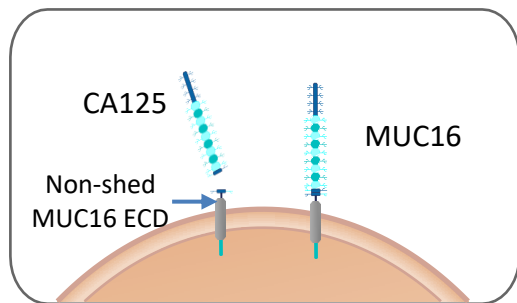
David Dornan, PhD

I have the following relevant financial relationships to disclose:

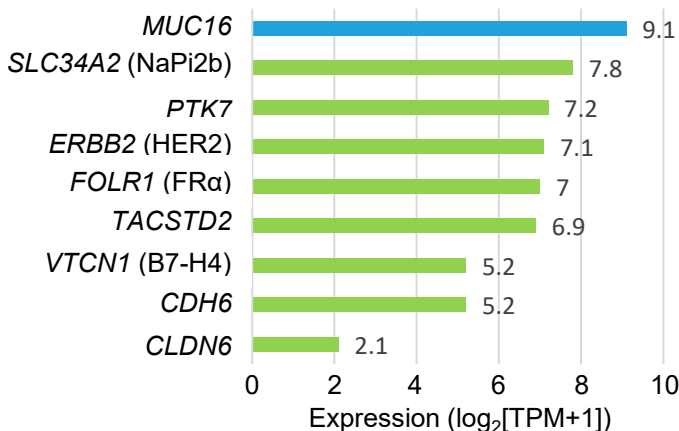
Employee of Whitehawk Therapeutics

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MUC16 as a Target for ADCs in Ovarian Cancer



MUC16 Expression Relative to Other ADC Targets in OC⁴



MUC16

- MUC16 is a heavily glycosylated cell-surface protein that plays a key role in cancer progression by facilitating tumor cell proliferation, invasion, and immune evasion^{1,2}
- CA125, the well-known cancer antigen and biomarker, is a proteolytic cleavage product of MUC16 that is shed into the blood stream. Non-shed MUC16 extracellular domain (ECD) remains on the tumor cell surface^{1,2}

MUC16 Expression in Cancer

- MUC16 is highly expressed in multiple tumor types, including ovarian, endometrial, cervical, pancreatic, and lung cancers, and has relatively low expression in normal tissues¹⁻³
- In ovarian cancer (OC), MUC16 expression is 2- to >100-fold higher than that of clinically validated and emerging ADC targets⁴

A Validated Target in Heavily Pretreated Platinum-Resistant OC^{5,6}

- Previous ADCs targeting MUC16 (DMUC4064A and DMUC5754A) demonstrated response rates of 14% to 42% in patients with high MUC16 expression^{5,6}
 - Higher MUC16 expression correlated with greater clinical activity^{5,6}
- Common TRAEs included fatigue, nausea, and peripheral neuropathy; grade ≥3 TRAEs included neutropenia, fatigue, and ocular toxicity^{5,6}
- However, since these ADCs bound shed CA125, higher dosing was required to overcome target-mediated drug disposition⁵⁻⁸

HWK-016 Is an Optimized ADC with Improved Tumor Targeting and Next-Generation Linker–Payload

Targeting

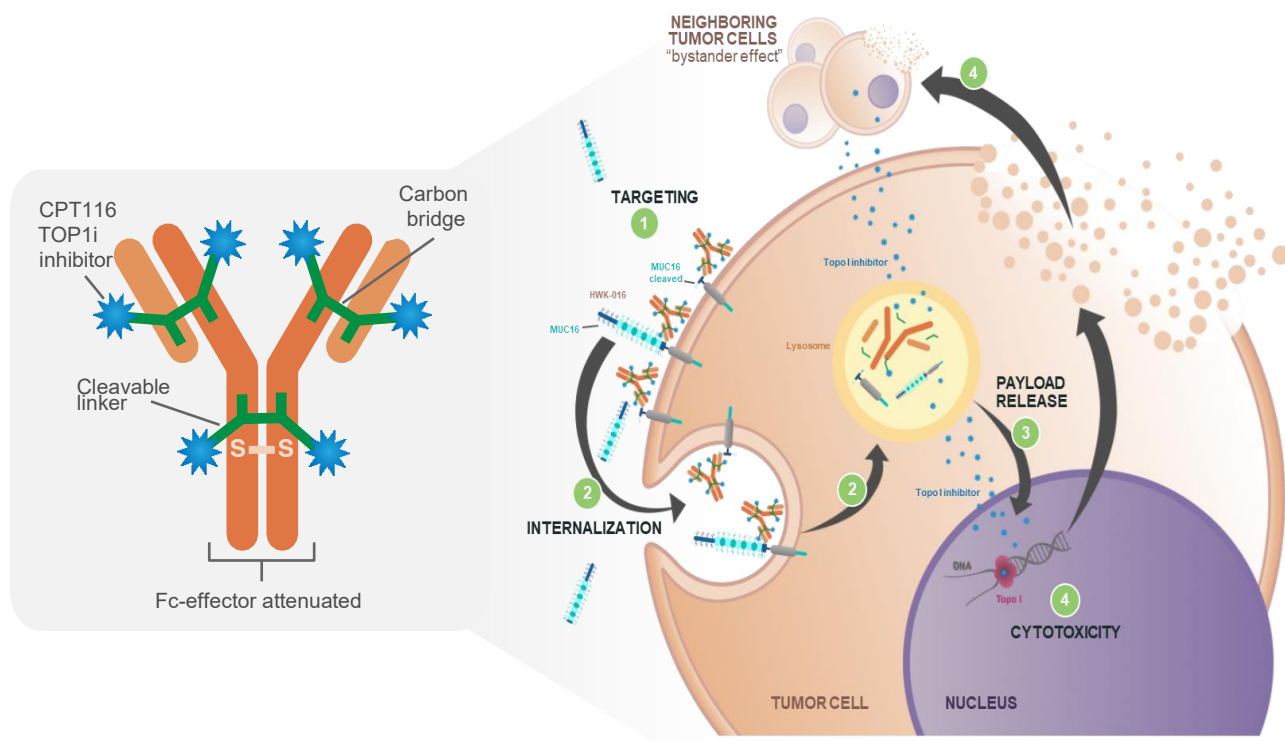
- Antibody targeting membrane-bound ECD of MUC16 for improved tumor targeting
- Attenuated Fc region

Bioconjugation

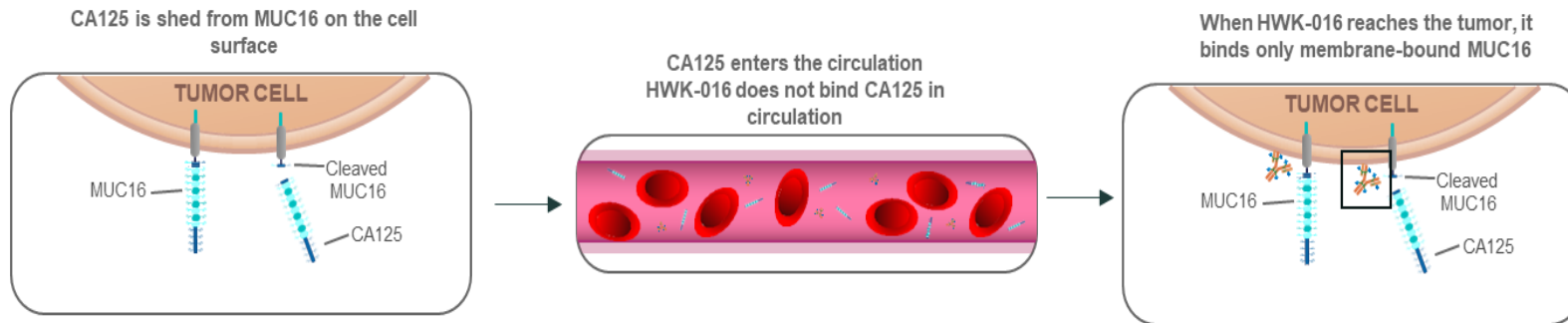
- Carbon bridge cysteine re-pairing
- Optimized DAR6 configuration
- Stability-enhancing hydrolysis

Linker-Payload (CPT113)

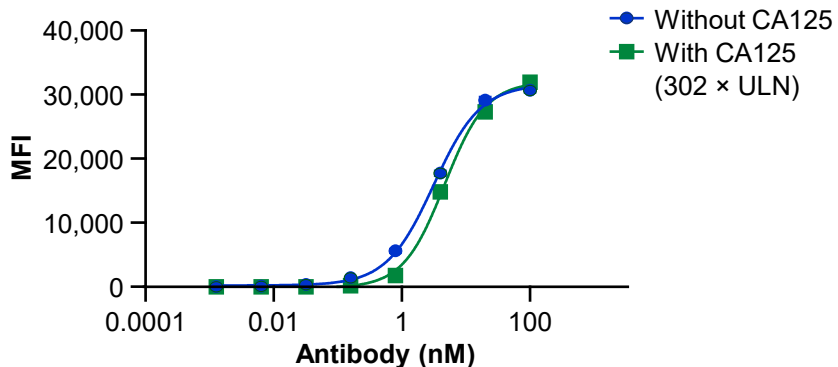
- PEG masking
- Intracellular triple peptide cleavage site
- Proprietary TOP1i payload



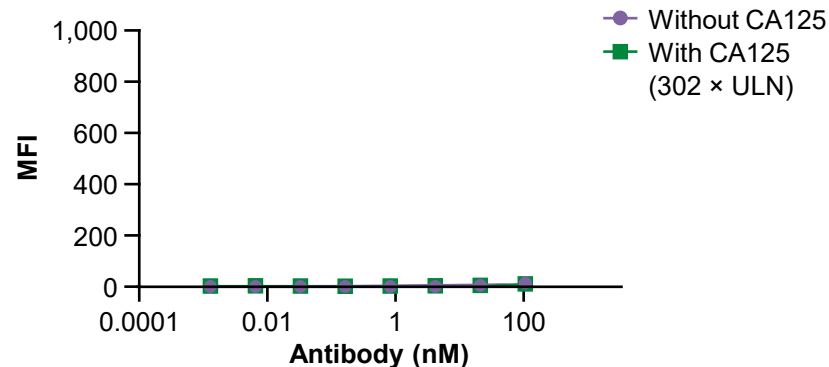
HWK-016 Targets Non-Shed, Extracellular Domain (ECD) MUC16 Rather than Cleavage Product, CA125



HWK-016 mAb Binding (MUC16 ECD Engineered SK-OV-3)

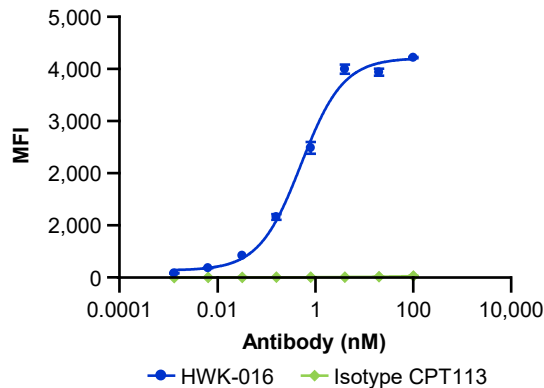


DMUC5457A mAb Binding (MUC16 ECD Engineered SK-OV-3)

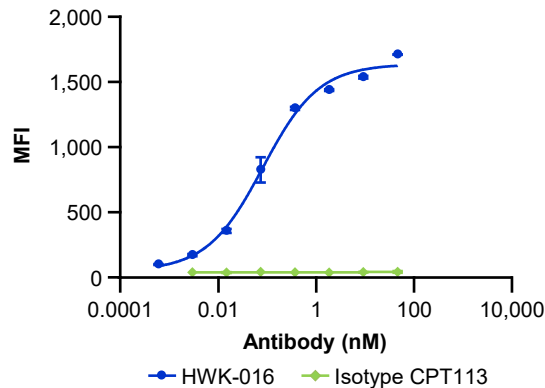


HWK-016 Binds to Cell Lines Expressing Varying Levels of MUC16

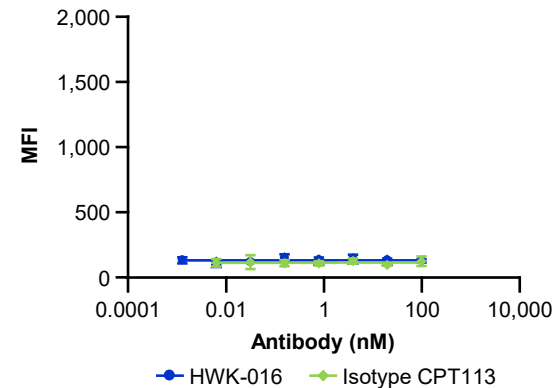
OVCAR-3 (OV, MUC16^{high})



SW1990 (PAAD, MUC16^{medium})



SK-OV-3 (OV, MUC16^{low})

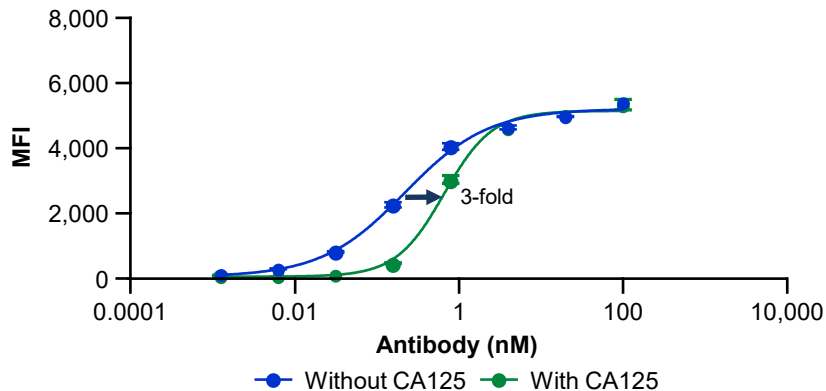


Cell Line	HWK-016		Isotype-CPT113	
	EC ₅₀ (nM)	Max MFI	EC ₅₀ (nM)	Max MFI
OVCAR-3 (MUC16 ^{high})	0.51	4200	NA	39
SW1990 (MUC16 ^{medium})	0.18	1708	NA	52
SK-OV-3 (MUC16 ^{low})	NA	172	NA	159

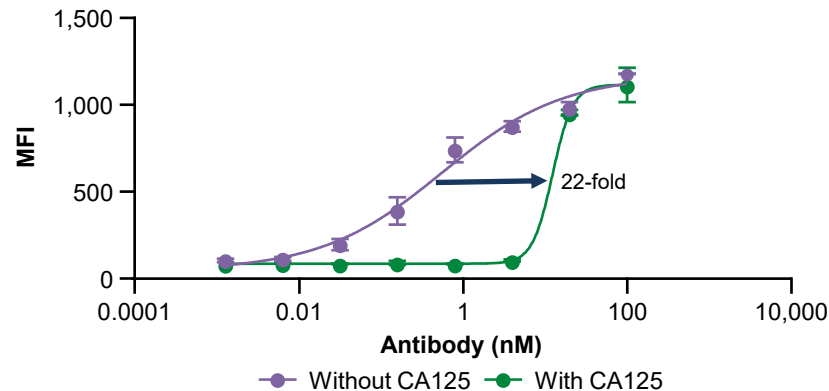
HWK-016 binding is dependent on MUC16 expression

HWK-016 mAb Binding to MUC16 on Cell Surface Is Minimally Impacted by Exogenous CA125

**HWK-016 mAb Binding
SW1990**



**DMUC5754A mAb Binding
SW1990**



CA125 Concentration	HWK-016 mAb EC ₅₀ (nM)	Fold Change
0 U/mL	0.22	N/A
10,623 U/mL (304 × ULN)	0.67	3.0

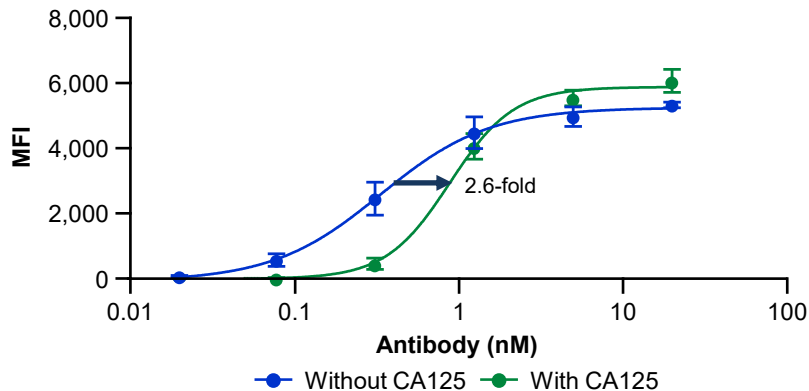
Note: In patients with cancer, high CA125 is defined as 2 × ULN (>70 U/mL).

CA125 Concentration	DMUC5754A mAb EC ₅₀ (nM)	Fold Change
0 U/mL	0.56	N/A
10,623 U/mL (304 × ULN)	12.4	22.2

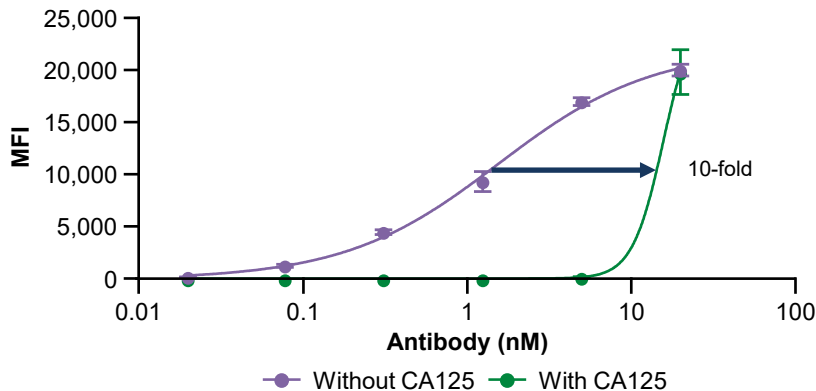
3-fold shift in EC₅₀ for HWK-016 mAb binding vs 22-fold shift in EC₅₀ for DMUC5754 mAb indicates minimal potential for high levels of CA125 to affect HWK-016 cell surface binding

HWK-016 mAb Internalization Is Minimally Impacted by Exogenous CA125

HWK-016 mAb Internalization SW1990



DMUC5754A mAb Internalization SW1990



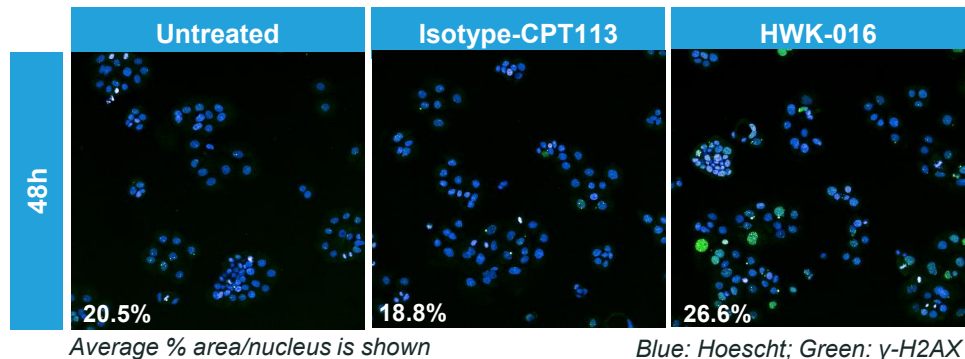
CA125 Concentration	HWK-016 mAb EC ₅₀ (nM)	Fold Change
0 U/mL	0.34	N/A
10,623 U/mL (304 × ULN)	0.89	2.6

CA125 Concentration	DMUC5754A mAb EC ₅₀ (nM)	Fold Change
0 U/mL	1.6	N/A
10,623 U/mL (304 × ULN)	~15.8	10.1

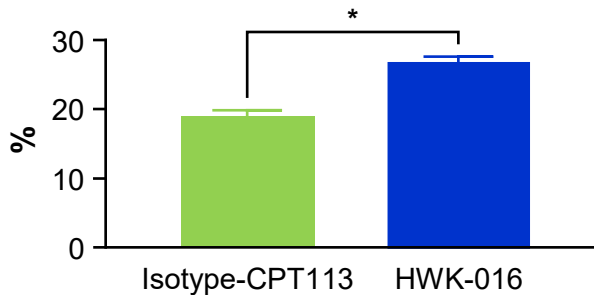
Note: In patients with cancer, high CA125 is defined as 2 × ULN (>70 U/mL).

2.6-fold shift in EC₅₀ for HWK-016 mAb internalization vs 10-fold shift in EC₅₀ for DMUC5754 mAb indicates minimal potential for high levels of CA125 to affect HWK-016-mediated cancer cell internalization

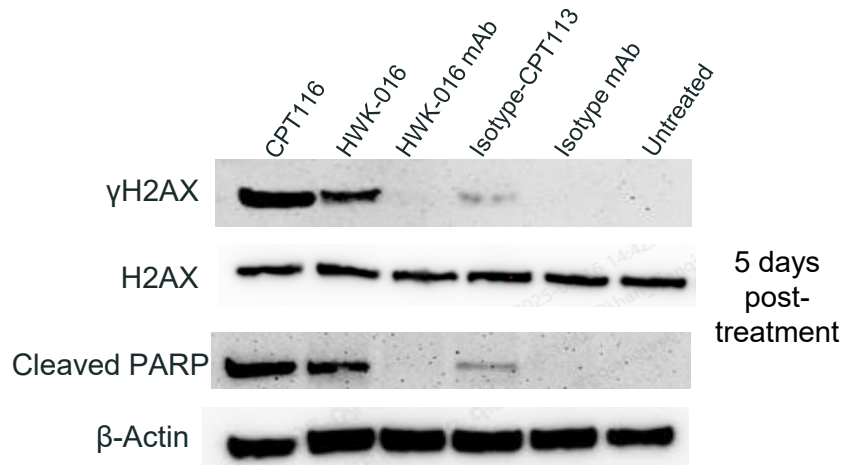
HWK-016 Induces DNA Damage and Apoptosis in OVCAR-3



γ -H2AX Activation by HWK-016 (% Area / Nucleus)

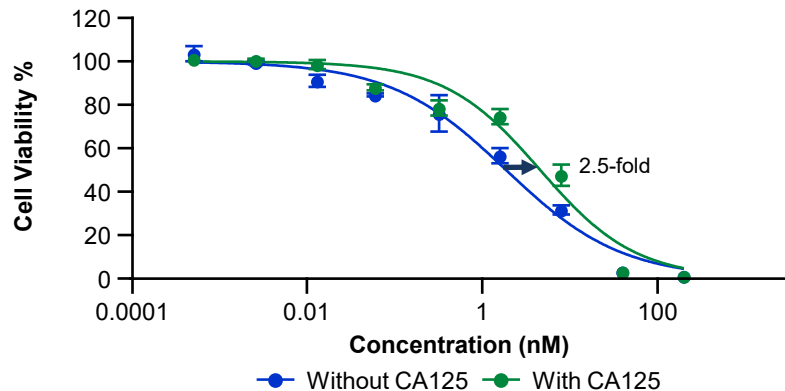


*, $p < 0.05$, paired t-test

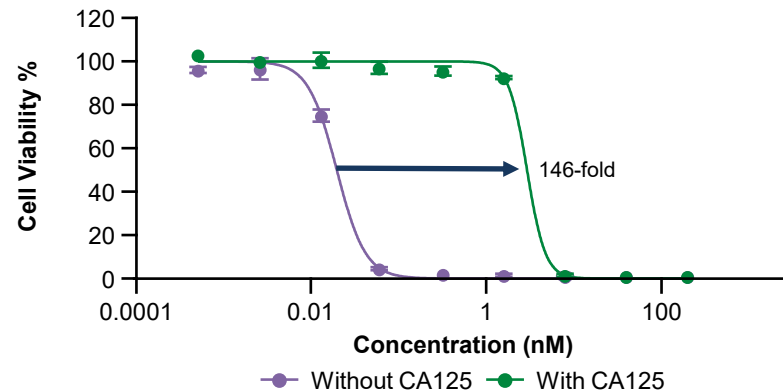


HWK-016–Mediated Inhibition of Cell Viability Is Minimally Impacted by Exogenous CA125

**HWK-016 Cell Viability
OVCAR-3**



**DMUC5754A-mAb-CPT113 Cell Viability
OVCAR-3**



CA125 Concentration	HWK-016 mAb EC ₅₀ (nM)	Fold Change
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0 U/mL	1.86	N/A
10,623 U/mL (304× ULN)	4.57	2.5

Note: In patients with cancer, high CA125 is defined as 2× ULN (>70 U/mL).

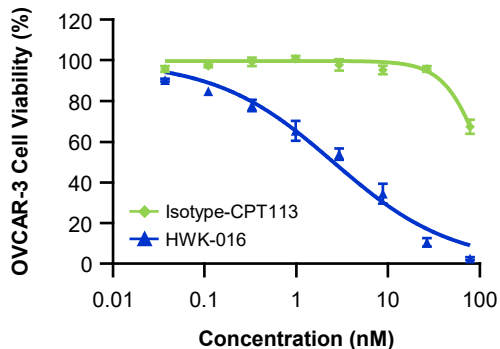
CA125 Concentration	DMUC5754A-mAb-CPT113 EC ₅₀ (nM)	Fold Change
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0 U/mL	0.02	N/A
10,623 U/mL (304× ULN)	2.93	146.5

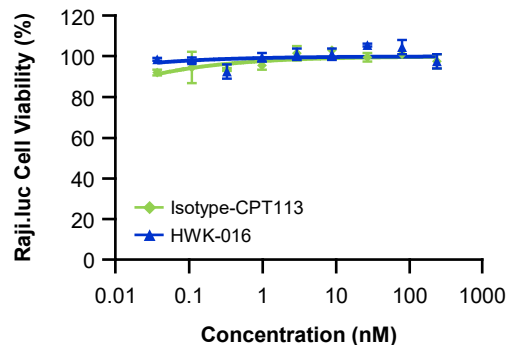
2.5-fold shift in EC₅₀ for HWK-016 induced inhibition of cell viability vs 146-fold shift in EC₅₀ for DMUC5754A-mAb-CPT113 indicates minimal potential for high levels of CA125 to affect HWK-016-mediated cancer cell killing

HWK-016 Reduced Viability of MUC16^{negative} Cells After Co-Culture with MUC16^{positive} Cells Demonstrating Bystander Effect

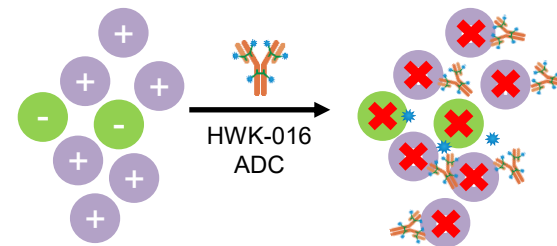
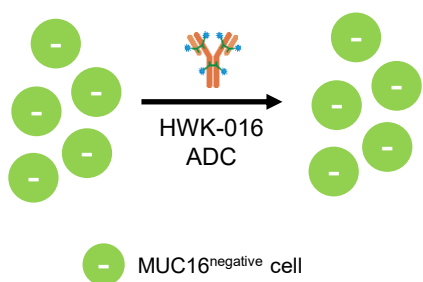
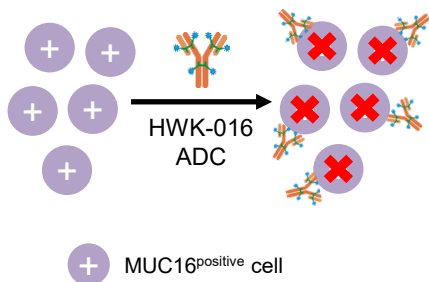
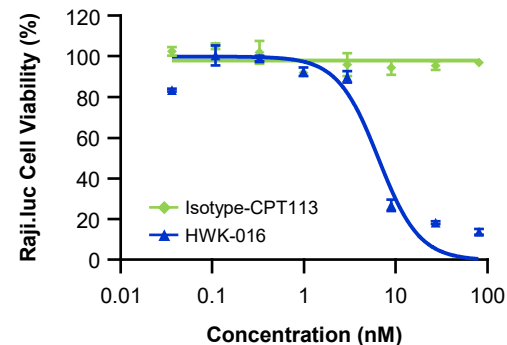
HWK-016
MUC16^{positive} OVCAR-3



HWK-016
MUC16^{negative} Raji-Luc

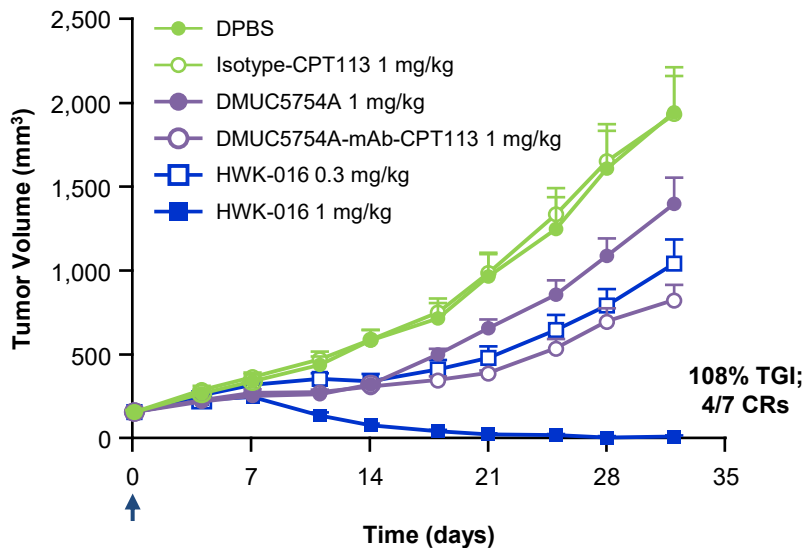


HWK-016
3:1 Co-Culture OVCAR-3:Raji-Luc



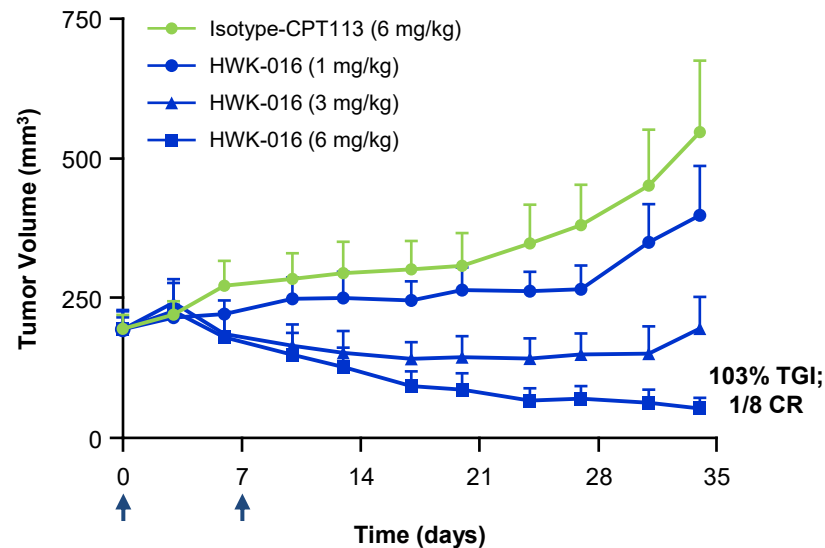
HWK-016 Induces Tumor Regression in Ovarian Cancer CDX and PDX Models

HWK-016 Efficacy in OVCAR-3 (MUC16^{high})



Note: OVCAR-3 sheds large amounts of CA125 (>600 ULN)

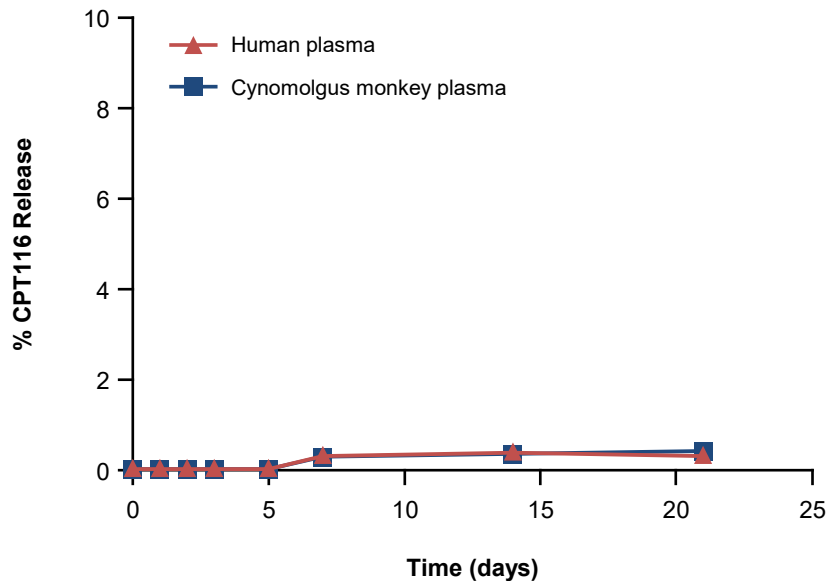
HWK-016 Efficacy in CTG-3423 (MUC16^{high})



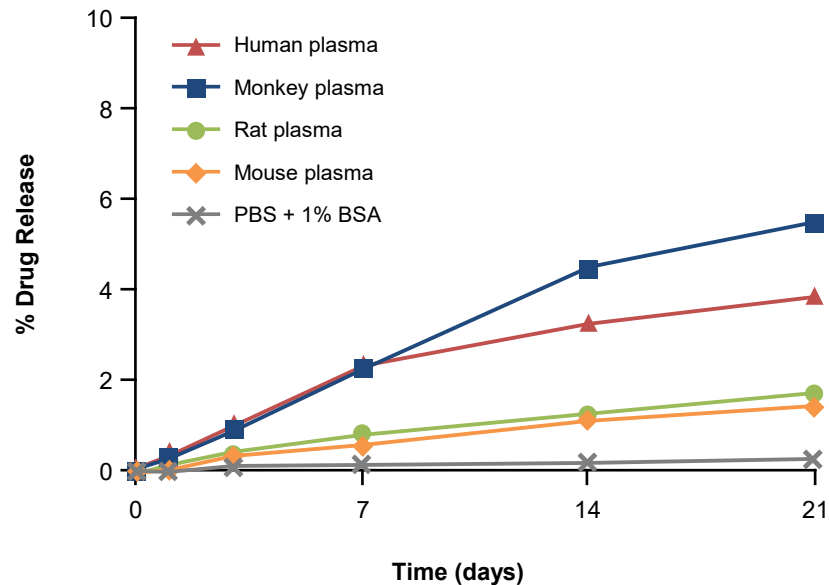
HWK-016 shows greater antitumor activity relative to DMUC5754A and DMUC5754A mAb conjugated to CPT113

HWK-016 Is Highly Stable in Human and Cynomolgus Monkey Plasma

HWK-016 In Vitro Plasma Stability



Dato-DXd In Vitro Plasma Stability¹

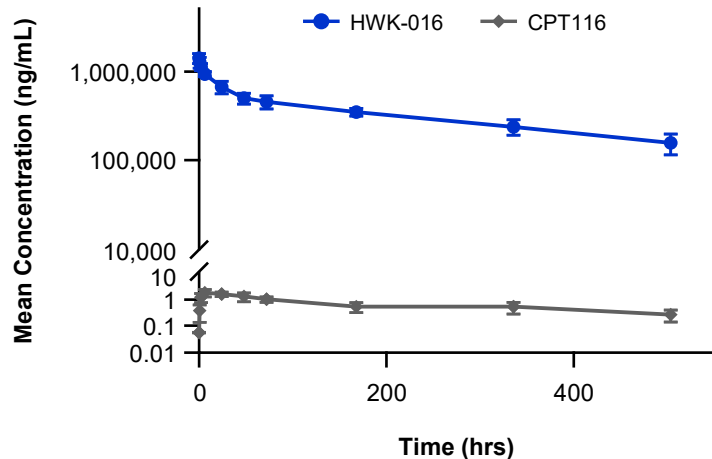


CPT116 release in human and cyno plasma is minimal ($\leq 0.4\%$)

HWK-016 Is Well Tolerated in Cynomolgus Monkeys and Demonstrates High Stability

Species	HNSTD	Findings
Cynomolgus monkey	60 mg/kg (highest dose tested)	No mortalities, all findings consistent with TOP1i payload class of ADC

HWK-016 60 mg/kg



- HWK-016 $T_{1/2}$ consistent with highly stable ADC (~10–13 days)
- Very low levels of CPT116 payload (<0.01%) released from HWK-016 into circulation

Conclusions

- HWK-016 is a promising next generation MUC16-targeting ADC that leverages novel bioconjugation and linker-payload technologies to minimize off-target effects and increase the therapeutic index
- HWK-016 selectively binds to the non-shed, ECD of MUC16 to ensure delivery to the tumor instead of circulating CA125
- HWK-016 demonstrated potent binding, internalization, and reduced cancer cell viability that are minimally impacted by addition of high levels of exogenous CA125
- HWK-016 has bystander activity and robust anti-tumor activity in models of ovarian cancer with tumor regressions observed at doses as low as 1 mg/kg
- In a repeat dose toxicology study, HWK-016 was well tolerated with an HNSTD of 60 mg/kg, the highest dose tested, and minimal levels of released payload were observed
- HWK-016 is currently being evaluated in a phase 1 study of patients living with advanced ovarian and endometrial cancers (NCT07470853)