Preclinical Assessment of HWK-007, a Next-Generation, PTK7-Targeting ADC with Novel Bioconjugation and Linker-Payload Technology

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Objective

The aim of these experiments was to characterize the mechanism of action of HWK-007, a next-generation, PTK7-targeting ADC, and to evaluate its suitability for clinical development

Conclusions

- **HWK-007 is a promising next-generation, PTK7-targeting ADC that leverages novel bioconjugation and linker-payload technologies to minimize off-target effects and increase therapeutic index versus prior PTK7-targeting ADCs**
- **HWK-007 demonstrates potent binding, internalization, and induction of DNA damage, leading to loss of cell viability in a range of solid cancer cell lines**
- **HWK-007 shows bystander activity and robust antitumor activity in SCLC and ovarian cancer models, with tumor regressions and complete responses observed at doses as low as 1 mg/kg**
- **HWK-007 is stable in cynomolgus monkey and human plasma**
- **In a repeat-dose toxicology study, HWK-007 was well tolerated with an HNSTD of 60 mg/kg (the maximal tested dose) and minimal levels of released free payload**
- **HWK-007 is currently being evaluated in a phase 1 clinical trial in patients with non-squamous, *EGFR*–wild-type NSCLC, platinum-resistant ovarian cancer, and endometrial cancer (NCT07444814)**

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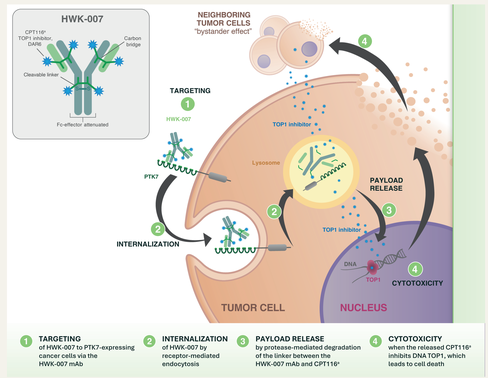
Kathleen S. Keegan is employed by and holds stock or stock options in Whitehawk Therapeutics.
For disclosures of co-authors, please refer to the abstract.

Background

- Protein tyrosine kinase-7 (PTK7) is a catalytically inactive receptor tyrosine kinase that promotes tumor progression through multiple signaling pathways. Overexpression of *PTK7* contributes to metastasis, chemoresistance, and poor clinical outcomes in multiple tumor types[1,2]
- PKT7 is a known clinical target of interest with a wide expression profile across numerous malignancies[1–3]
- Early-phase studies of the first-generation, PTK7-targeting, auristatin-based antibody-drug conjugate (ADC) cofetuzumab pelidotin showed promising signs of clinical activity[4,5]
- HWK-007 is a next-generation, PTK7-targeting ADC comprising a high-affinity, anti-PTK7 monoclonal IgG1 antibody with attenuated Fc activity conjugated to a novel topoisomerase I (TOP1) inhibitor payload, CPT116, via carbon-bridge cysteine re-pairing (**Figure 1**)
- HWK-007 leverages novel bioconjugation and linker-payload technologies that reduce cellular uptake of free payload relative to exatecan to minimize off-target effects and increase the therapeutic index. Once inside the cell, CPT116 inhibition of TOP1 is similar to exatecan with reduced potential for efflux mediated by drug resistance pumps, MDR1/PGP and BCRP[6]

FIGURE 1. HWK-007 Structure and Mechanism of Action



[a]CPT116 (an exatecan analog) is the payload.
DAR, drug-to-antibody ratio; **Fc**, fragment crystallizable; **mAb**, monoclonal antibody; **PTK7**, protein tyrosine kinase-7; **TOP1**, topoisomerase I.

Methods

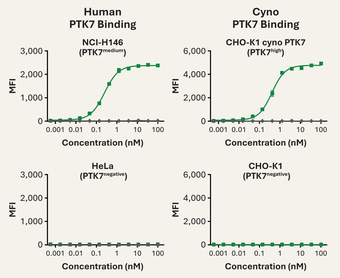
- HWK-007 binding affinity to human and cynomolgus monkey cells was determined using flow cytometry and surface plasmon resonance
- HWK-007 internalization was quantified using pHrodo red dye (Invitrogen) and analyzed by the PerkinElmer Operetta CLS High-Content Analysis system
- DNA damage was quantified by staining HWK-007–treated cells with anti–γ-H2AX antibodies and imaged using the Opera Phenix high-content imager (PerkinElmer)
- Cell viability was measured using the Cell Titer-Glo luminescent assay (Promega)

Results

HWK-007 BINDING AND INTERNALIZATION

- The HWK-007 monoclonal antibody (mAb) bound to human and cynomolgus monkey PTK7 with high affinity (nanomolar half-maximal effective concentration [EC_{50}]) (**Figure 2**)
  - The off-rate of HWK-007 mAb, as measured by surface plasmon resonance, was slower than that of cofetuzumab, a first-generation, PTK7-targeting mAb
- The HWK-007 ADC was selectively internalized by tumor cells across a wide range of concentrations (**Figure 3**)

FIGURE 2. HWK-007 mAb Binds with High Affinity to Human and Cyno PTK7



Binding Affinity of HWK-007 mAb to Human and Cyno PTK7 by Flow Cytometry

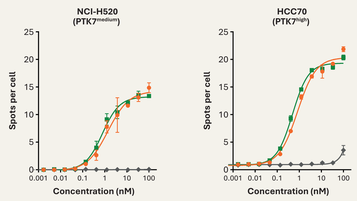
Cell line	HWK-007 mAb EC_{50} (nM)
NCI-H146 ($PTK7^{medium}$)	0.24
HeLa ($PTK7^{negative}$)	NA
CHO-K1 cyno PTK7 ($PTK7^{high}$)	0.4
CHO-K1 ($PTK7^{negative}$)	NA

Binding Affinity of HWK-007 mAb to Human and Cyno PTK7 by SPR

Antibody	Analyte	K_a (1/Ms)	K_d (1/s)	K_D (M)
HWK-007	Human PTK7	7.88×10^{6}	1.92×10^{-5}	2.44×10^{-10}
	Cyno PTK7	6.74×10^{6}	3.76×10^{-5}	5.58×10^{-10}
Cofetuzumab	Human PTK7	5.96×10^{6}	4.31×10^{-4}	7.23×10^{-9}
	Cyno PTK7	5.48×10^{6}	3.60×10^{-4}	6.57×10^{-9}

NCI-H146 (human PTK7-positive SCLC), HeLa (human PTK7-negative cervical adenocarcinoma), CHO-K1 cyno PTK7 (cyno PTK7-positive engineered cell line), and CHO-K1 (cyno PTK7-negative parental line) cells were incubated with HWK-007 mAb, cofetuzumab, or IgG1 isotype control mAb at concentrations of 5.6×10^{-4} nM to 100 nM for 1 h at 4°C. Antibody binding was measured using flow cytometry and analyzed using FlowJo; EC_{50} values were calculated using GraphPad Prism. For SPR, HWK-007 mAb or cofetuzumab was captured on an anti-human IgG, Fc-immobilized CM5 sensor chip (Cytiva). Recombinant human PTK7 ECD protein, cyno PTK7 ECD protein, and rat PTK7 ECD protein at concentrations ranging from 3.13–200 nM were injected over the sensor chip. Data were analyzed using BIAcore Insight Evaluation Software.
Cyno, cynomolgus monkey; **EC_{50}**, half-maximal effective concentration; **ECD**, extracellular domain; **K_a**, association constant; **K_d**, dissociation constant; **K_D**, equilibrium dissociation constant; **mAb**, monoclonal antibody; **MFI**, mean fluorescence intensity; **NA**, not applicable; **PTK7**, protein tyrosine kinase-7; **SCLC**, small cell lung cancer; **SPR**, surface plasmon resonance.

FIGURE 3. HWK-007 Internalizes into Cancer Cell Lines with Varying Levels of PTK7 Expression



Cell line	HWK-007		Cofetuzumab-Aur0101	
	EC_{50} (nM)	Maximum spots per cell	EC_{50} (nM)	Maximum spots per cell
NCI-H520 ($PTK7^{medium}$)	0.78	14	1.4	16
HCC70 ($PTK7^{high}$)	0.50	21	0.84	22

Cells were incubated at 37°C with 100 nM pHrodo-labeled HWK-007, cofetuzumab-Aur0101, or isotype-CPT113 (isotype-matched control ADC) and titrated via 4-fold dilution. pHrodo fluoresces at the low pH found in lysosomes, allowing tracking of ADC internalization. At 4 h, intensity per well and positive cells per well were measured and analyzed using the PerkinElmer Operetta CLS High-Content Analysis system. Data were analyzed using GraphPad Prism.
ADC, antibody-drug conjugate; **EC_{50}**, half-maximal effective concentration; **PTK7**, protein tyrosine kinase-7.

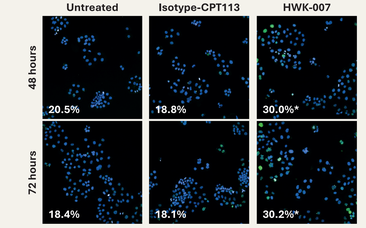
IN VITRO TUMOR ACTIVITY

- Consistent with its payload's mechanism of action, HWK-007 induced tumor cell DNA damage as measured by activation of γ-H2AX (**Figure 4**)
- HWK-007 demonstrated potent tumor cell killing across a range of solid tumor cell lines at 25- to >100-fold higher EC_{50} than cofetuzumab-Aur0101 (**Figure 5**)
- HWK-007 also showed substantial bystander killing, as indicated by experiments in which HWK-007 did not induce cell death in *PTK7*-negative tumor cells unless they were co-cultured with *PTK7*-expressing cells (**Figure 6**)

IN VIVO TUMOR ACTIVITY

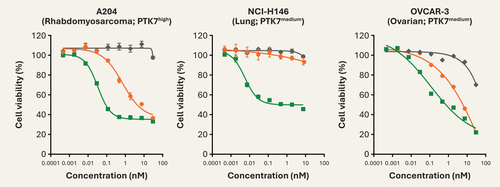
- HWK-007 showed robust antitumor activity in small cell lung cancer and ovarian cancer models with tumor growth inhibition ranging from 76% to 107% depending on the HWK-007 dose and tumor model (**Figure 7**)
- HWK-007 resulted in complete responses at doses as low as 1 mg/kg (**Figure 7**)

FIGURE 4. HWK-007 Induces DNA Damage in OVCAR-3 Cancer Cells



OVCAR-3 cells were incubated with 1.2 nM HWK-007 or isotype-CPT113 (isotype-matched control ADC) for 48 or 72 h, fixed, and permeabilized. They were then stained with mouse anti–γ-H2AX antibody (green), and counterstained with Hoechst (blue). Imaging was conducted using the Opera Phenix high-content imager (PerkinElmer). MFI from triplicate images is shown. Statistical significance was determined using multiple unpaired t tests in GraphPad Prism 7. *p<0.05.
ADC, antibody-drug conjugate; **MFI**, mean fluorescence intensity.

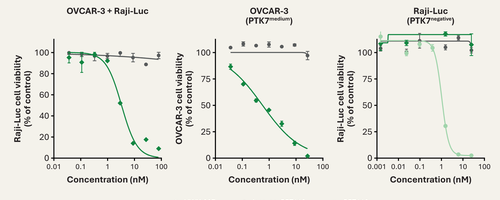
FIGURE 5. HWK-007 Induces Cancer Cell Death Across Cancer Cell Lines with Greater Potency Than Cofetuzumab-Aur0101



Cell line	HWK-007		Cofetuzumab-Aur0101	
	EC_{50} (nM)	E_{max} (nM)	EC_{50} (nM)	E_{max} (nM)
A204 ($PTK7^{high}$)	0.03	67	0.71	64
NCI-H146 ($PTK7^{medium}$)	0.006	56	NA	10
OVCAR-3 ($PTK7^{medium}$)	0.12	78	81	78

A204, NCI-H146, or OVCAR-3 cells were incubated with HWK-007, cofetuzumab-Aur0101, or isotype-CPT113 (isotype-matched control ADC) at concentrations ranging from 4.6×10^{-4} nM to 30 nM at 37°C continuously for 6 or 7 days. Cell viability was assessed using the Cell Titer-Glo luminescent assay (Promega).
ADC, antibody-drug conjugate; **EC_{50}**, half-maximal effective concentration; **E_{max}**, maximal effect; **NA**, not applicable.

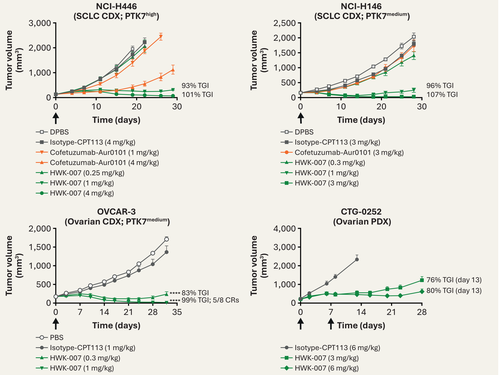
FIGURE 6. HWK-007 Demonstrates Potent Bystander Killing of *PTK7*-Negative Cancer Cells



	OVCAR-3 + Raji-Luc (Ratio = 3:1)		OVCAR-3 ($PTK7^{medium}$)		Raji-Luc ($PTK7^{negative}$)	
	IC_{50} (nM)	E_{max} (%)	IC_{50} (nM)	E_{max} (%)	IC_{50} (nM)	E_{max} (%)
HWK-007	2.9	91	1.4	100	>100	NA
Isotype-CPT113	>100	NA	>100	NA	>100	NA
CPT116	ND	NA	ND	NA	1.05	100

HWK-007, isotype-CPT113 (isotype-matched control ADC), or CPT116 were incubated for 10 days at 37°C with OVCAR-3 (an ovarian cell line endogenously expressing *PTK7*), Raji-Luc (a *PTK7*-negative cell line expressing luciferase), or a co-culture of the two cell lines. Cell viability was determined using Cell Titer-Glo (Promega) or Bright-Lite Luciferase Assay (Vazyme) and detected by Envision. Data were analyzed using GraphPad Prism 7. HWK-007 demonstrated cell killing in a ligand-dependent manner in OVCAR-3 cells, but not in Raji-Luc cells. In the co-culture experiment, Raji-Luc cells incubated with HWK-007 were killed, whereas those incubated with isotype-matched control ADC were not. These data suggest that uptake of HWK-007 into OVCAR-3 cells led to payload release and cell killing of Raji-Luc through bystander activity.
ADC, antibody-drug conjugate; **E_{max}**, maximal effect; **IC_{50}**, half-maximal inhibitory concentration; **NA**, not applicable.

FIGURE 7. HWK-007 Shows Robust Antitumor Activity in SCLC and Ovarian Cancer Models

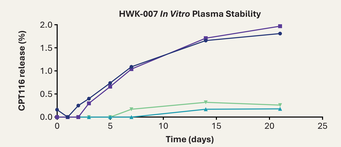


Antitumor activity of HWK-007 was evaluated in ovarian (OVCAR-3) and SCLC (NCI-H446, NCI-H146) CDX models, and in an ovarian PDX model (CTG-0252). In the CDX models, a single intravenous dose of HWK-007 (0.3–3 mg/kg for OVCAR-3 and NCI-H146, or 0.25–4 mg/kg for NCI-H446), cofetuzumab-Aur0101(1–4 mg/kg) or isotype-CPT113 (isotype-matched control ADC) was administered to eight mice in each group for NCI-H446; five in each group for NCI-H146; and eight in each group for OVCAR-3 after tumors had grown to 125–150 mm^3 (day 0), as indicated by the arrow. In the CTG-0252 model, two doses of 3 or 6 mg/kg HWK-007 or 6 mg/kg isotype-CPT113 were administered after tumors had grown to 200 mm^3 (day 0) and on day 7, as indicated by the arrows. Percentage TGI was calculated as TGI (%) = $[1-(T_f-T_0)/(V_f-V_0)]\times100\%$, in which T_f = volume of treatment group on day i after administration; T_0 = mean tumor volume of treatment group on day 0 after administration; V_f = mean tumor volume of negative control group on day i after administration; and V_0 = mean tumor volume of negative control group on day 0. Data were analyzed by one-way ANOVA and two-way ANOVA using GraphPad Prism 6. ****p<0.00001.
ANOVA, analysis of variance; **CDX**, cell line-derived xenograft; **CR**, complete response; **DPBS**, Dulbecco's phosphate-buffered saline; **PBS**, phosphate-buffered saline; **PDX**, patient-derived xenograft; **SCLC**, small cell lung cancer; **TGI**, tumor growth inhibition.

PHARMACOKINETICS

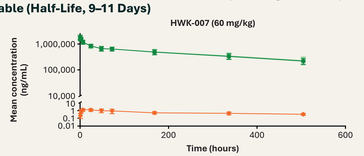
- HWK-007 was stable in human and cynomolgus monkey plasma *in vitro*, with minimal (<0.05%) release of free CPT116 payload over 21 days (**Figure 8**)
- Plasma levels of HWK-007 ADC were stable with a half-life of 9–11 days in cynomolgus monkeys treated with HWK-007. Very low levels of free CPT116 payload (0.0067%) were detected (**Figure 9**)
- Consistent with this, the highest non-severely toxic dose (HNSTD) of HWK-007 was considered to be 60 mg/kg; this was the highest dose tested in a study in which cynomolgus monkeys were administered intravenous HWK-007 once every 3 weeks for 22 days (two doses in total) with a 28-day recovery period

FIGURE 8. HWK-007 Is Stable in Human and Cyno Plasma with Low Free Payload Release



HWK-007 was incubated with plasma at 37°C for 21 days. At each timepoint, samples were frozen with liquid nitrogen and stored at –80°C. Released free payload (CPT116) was quantified using triple quadrupole liquid chromatography with tandem mass spectrometry.
Cyno, cynomolgus monkey.

FIGURE 9. HWK-007 Is Well Tolerated in Cynomolgus Monkeys and Is Highly Stable (Half-Life, 9–11 Days)



HWK-007, total antibody, or free payload (CPT116) was administered as a single dose. Plasma was collected from time 0 to 504 h and purified. HWK-007 and total antibody concentrations were assessed using enzyme-linked immunosorbent assay. Free CPT116 payload was quantified using triple quadrupole liquid chromatography with tandem mass spectrometry.

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