Preclinical Assessment of HWK-206, a Next-Generation, Biparatopic, SEZ6-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 and antitumor activity of HWK-206, a biparatopic, SEZ6-targeting ADC, and determine its suitability for clinical development

Conclusions

» HWK-206 is a promising next-generation, SEZ6-targeting ADC that leverages novel bioconjugation and linker-payload technologies to minimize off-target effects and increase therapeutic index versus other SEZ6-targeting ADCs

» The bipratropic, SEZ6-targeting HWK-206 antibody demonstrates increased binding and internalization compared with parental mAbs alone or ABBV-706—a SEZ6-targeting ADC in clinical development

» HWK-206 shows greater inhibition of cell viability compared with ABBV-706 in cell lines with varying SEZ6 expression

» *In vivo*, HWK-206 demonstrates potent antitumor activity and improved activity compared with ABBV-706 mAb conjugated to CPT113

» HWK-206 is stable *in vitro* when incubated with human plasma and *in vivo* after dosing in cynomolgus monkeys

» In repeat-dose toxicology studies, HWK-206 was well tolerated with an HNSTD of 60 mg/kg (the maximal tested dose), and minimal levels of free payload were detected

» Investigational New Drug filing is planned for mid-2026 and initiation of a phase 1 trial is expected Q3 2026

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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

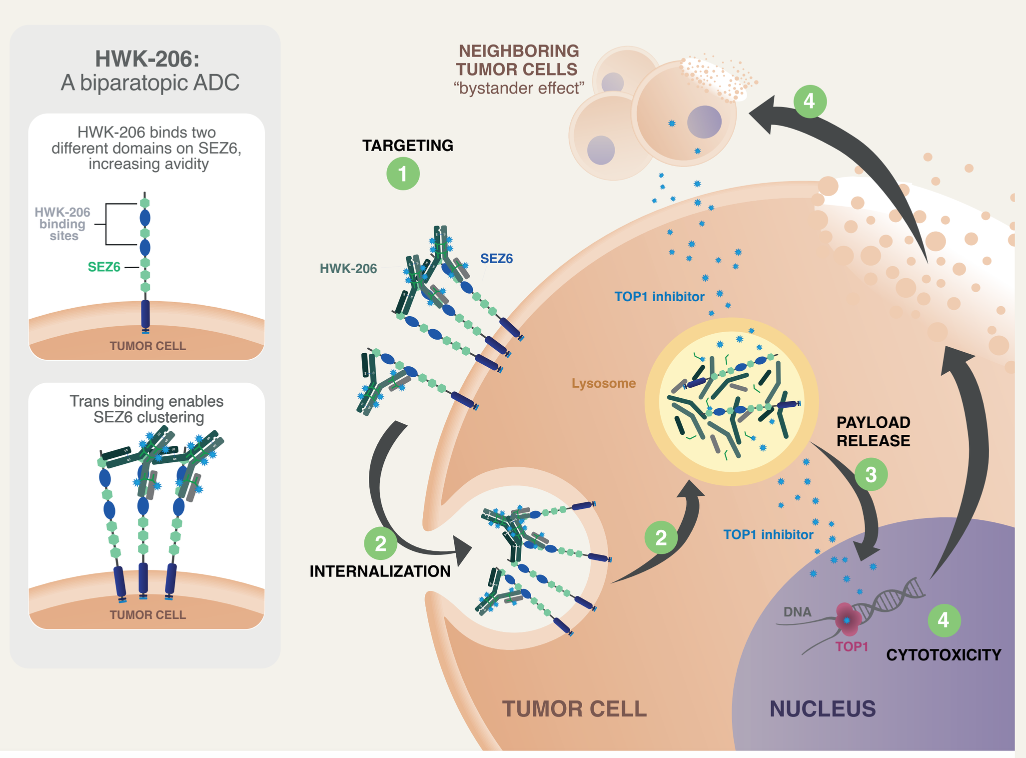
» Seizure-related homolog protein 6 (SEZ6) is a transmembrane protein involved in neuronal development.[1] It is overexpressed in various solid tumors, including small cell lung cancer (SCLC), neuroendocrine neoplasms (NENs), and central nervous system tumors, and has limited expression in normal tissues.[2] In SCLC, SEZ6 expression is correlated with poor survival outcomes[3]

» SEZ6-targeted antibody-drug conjugates (ADCs), such as ABBV-706, have shown initial activity in SCLC and NENs, validating SEZ6 as a promising ADC target[2,4]

» HWK-206 is a next-generation ADC that targets SEZ6 utilizing a biparatopic antibody (bpAb) with attenuated Fc activity, resulting in improved binding and internalization to deliver more payload to the target versus other SEZ6-targeting ADCs (**Figure 1**)

» The HWK-206 linker-payload technology comprises a proprietary DNA topoisomerase I (TOP1) inhibitor, CPT116, using novel bioconjugation (carbon bridge cysteine re-pairing) and advanced linker-payload technologies to maximize intracellular delivery while minimizing systemic exposure of free payload (**Figure 1**)

FIGURE 1. HWK-206 Mechanism of Action



1. **TARGETING** HWK-206 is a biparatopic ADC that binds two distinct domains on SEZ6; this trans binding enables increased avidity and clustering of SEZ6
2. **INTERNALIZATION** of HWK-206 is rapid and pronounced
3. **PAYLOAD RELEASE** by protease-mediated degradation of the linker between the HWK-206 bpAb and CPT116
4. **CYTOTOXICITY** when the released CPT116 inhibits DNA TOP1, which leads to cell death

ADC, antibody-drug conjugate; **bpAb**, biparatropic antibody; **CPT116**, an exatecan analog; **SEZ6**, seizure-related homolog protein 6; **TOP1**, topoisomerase I.

Methods

» HWK-206 binding affinity to SHP77.hSEZ6^{high} (SCLC) cells was determined using flow cytometry and surface plasmon resonance

» HWK-206 internalization was quantified using pHrodo red dye (Invitrogen) and analyzed using the PerkinElmer Operetta CLS High-Content Analysis system

» DNA damage was quantified by staining HWK-206–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

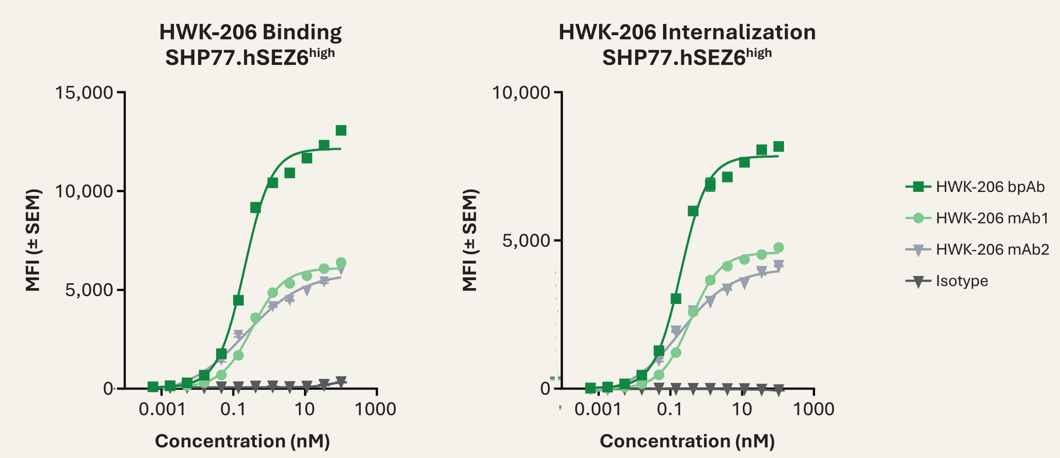
HWK-206 BINDING AND INTERNALIZATION

» The HWK-206 bpAb bound to and was internalized by SEZ6-expressing SCLC cells with nanomolar half-maximal effective concentrations (EC_{50}) (**Figure 2**)

– Higher maximal binding and higher internalization were observed with HWK-206 bpAb compared with either parent monoclonal antibody (mAb) alone

» HWK-206 bpAb bound with high affinity to cell lines with varying levels of SEZ6 expression and with higher affinity and maximal binding than the ABBV-706 mAb—the SEZ6-targeting mAb component of ABBV-706, a SEZ6-targeting ADC in clinical development (**Figure 3**)

FIGURE 2. HWK-206 bpAb Binds with High Affinity to SEZ6-Expressing SCLC Cells and Is Internalized to a Greater Degree Than Either Parent mAb

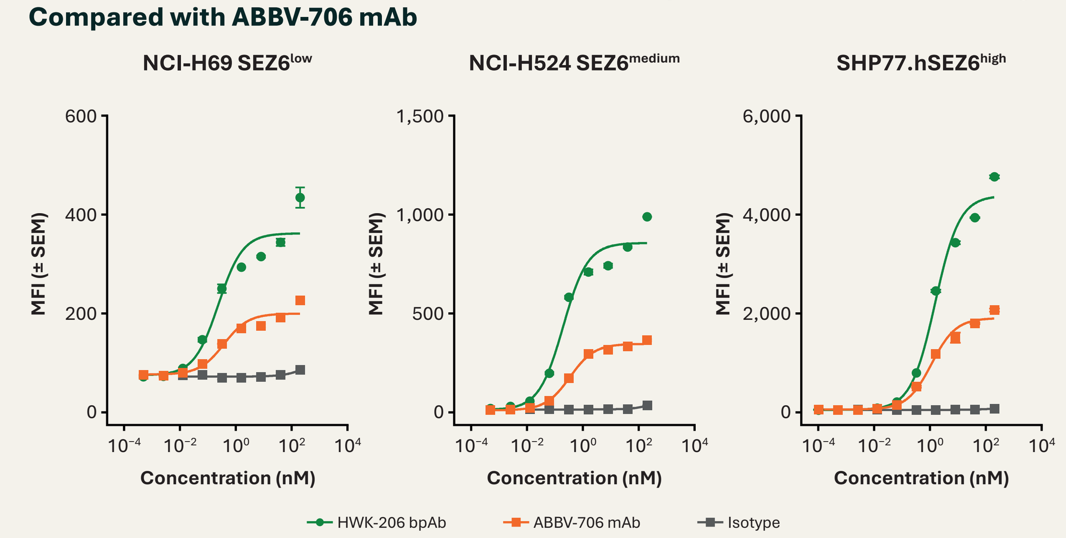


Antibody	Binding		Internalization	
	EC_{50} (nM)	Maximum MFI	EC_{50} (nM)	Maximum MFI
HWK-206 bpAb	0.20	12,130	0.19	7,835
HWK-206 mAb1	0.34	6,098	0.34	4,592
HWK-206 mAb2	0.21	5,854	0.18	4,047

Cell surface binding was assessed by incubating SHP77.hSEZ6^{high} cells (an SCLC cell line engineered to express high levels of human SEZ6) with the HWK-206 bpAb, either parent antibody (HWK-206 mAb1, HWK-206 mAb2), or an isotype-matched control mAb at concentrations of 0.0005–200 nM for 1 h at 4°C. Antibody binding was measured using flow cytometry, analyzed using FlowJo, and EC_{50} values were calculated using GraphPad Prism 7. Internalization was assessed by incubating SHP77.hSEZ6^{high} cells with the HWK-206 bpAb, HWK-206 mAb1, HWK-206 mAb2, or an isotype-matched control mAb at concentrations of 0.0005–1000 nM for 1 h at 4°C. Bound antibody was labeled with AF647 goat anti-human IgG prior to incubation for 2 h at 37°C or 4°C (control). Cell surface-bound proteins were stripped using paraformaldehyde, and remaining fluorescence was measured using flow cytometry and analyzed by FlowJo. The EC_{50} values were calculated using GraphPad Prism 7.

bpAb, biparatopic antibody; **EC_{50}**, half-maximal effective concentration; **mAb**, monoclonal antibody; **MFI**, mean fluorescence intensity; **SCLC**, small cell lung cancer; **SEM**, standard error of the mean; **SEZ6**, seizure-related homolog protein 6.

FIGURE 3. HWK-206 bpAb Binds with High Affinity to Cancer Cells with Varying Levels of SEZ6 Expression and with Greater Affinity as well as Maximal Binding Compared with ABBV-706 mAb



Antibody	NCI-H69 SEZ6^{low}		NCI-H524 SEZ6^{medium}		SHP77.hSEZ6^{high}	
	EC_{50} (nM)	Maximum MFI	EC_{50} (nM)	Maximum MFI	EC_{50} (nM)	Maximum MFI
HWK-206 bpAb	0.24	363	0.20	859	1.5	4,383
ABBV-706 mAb	0.38	200	0.35	347	1.1	1,905

Cell surface binding was assessed by incubating cell lines of varying levels of SEZ6 expression—NCI-H69 SEZ6^{low}, NCI-H524 SEZ6^{medium}, and SHP77.hSEZ6^{high}—with HWK-206 bpAb, ABBV-706 mAb, or an isotype-matched control mAb at concentrations of 0.0005–200 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.

bpAb, biparatopic antibody; **EC_{50}**, half-maximal effective concentration; **mAb**, monoclonal antibody; **MFI**, mean fluorescence intensity; **SEM**, standard error of the mean; **SEZ6**, seizure-related homolog protein 6.

IN VITRO TUMOR ACTIVITY

» Consistent with its payload's mechanism of action, HWK-206 induced tumor cell DNA damage as measured by activation of γ-H2AX (**Figure 4**)

» CPT116 has been optimized to retain potent TOP1 inhibition and to reduce efflux and free payload release from the ADC

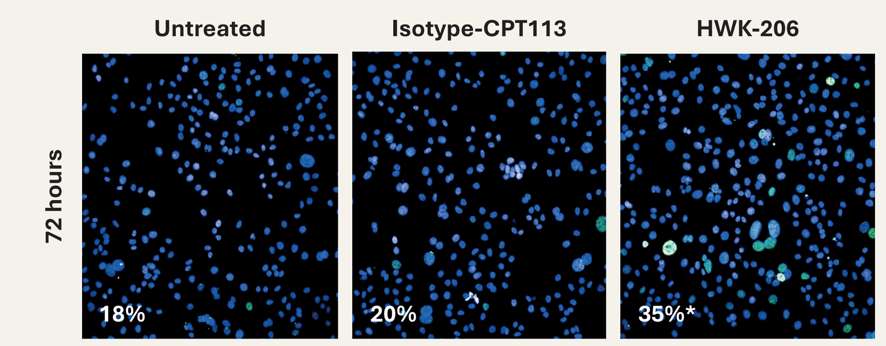
– *In vitro* TOP1 inhibition with CPT116 was similar to that of exatecan (half-maximal inhibitory concentration [IC_{50}] 1.8 μM vs 1.2 μM, respectively)

– CPT116 was 5.2- to 13.5-fold less potent than exatecan at inhibiting cell viability *in vitro* (**Table 1**)

– CPT116 is not a substrate for PGP/MDR1 or BCRP, minimizing the potential for efflux from cancer cells

» HWK-206 demonstrated potent tumor cell killing across a range of solid tumor cell lines with varying degrees of SEZ6 expression, and at 2- to 77-fold lower IC_{50} than ABBV-706 (**Figure 5**)

FIGURE 4. HWK-206 Induces DNA Damage in SCLC Cells



HCC1833 (SCLC) cells were incubated with 1.2 nM HWK-206 or isotype-CPT113 (isotype-matched control ADC) for 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. *$p<0.05$.

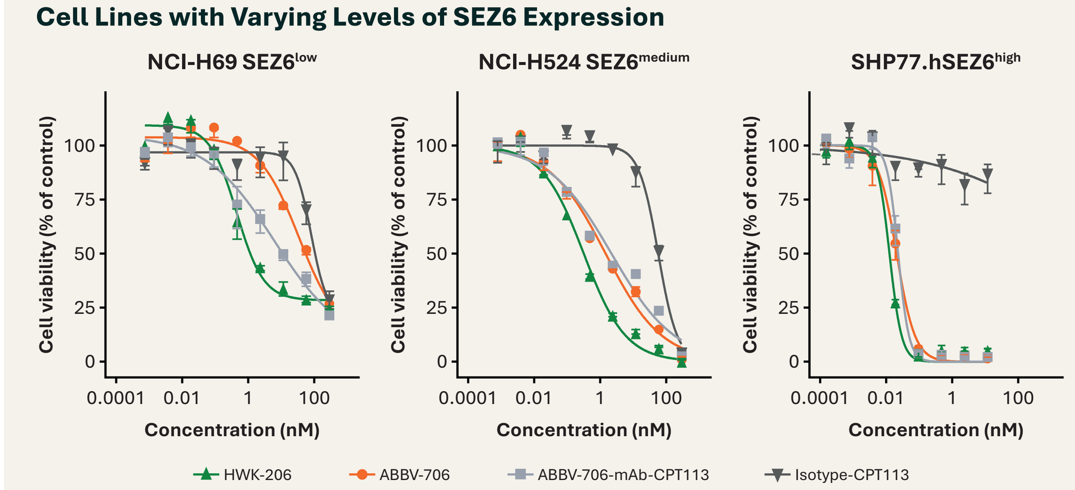
ADC, antibody-drug conjugate; **MFI**, mean fluorescence intensity; **SCLC**, small cell lung cancer.

TABLE 1. *In Vitro* Cell Viability Inhibition with CPT116 and Exatecan

TOP1 inhibitor	A204	OVCAR-3	NCI-H520	NCI-H1650	NCI-H441	NCI-H146
CPT116 (IC_{50} [nM])	3.2	0.67	4.6	19	2.5	0.39
Exatecan (IC_{50} [nM])	0.54	0.13	0.46	1.4	0.29	0.07
Fold potency (CPT116:exatecan)	5.9	5.2	10	14	8.6	5.5

IC_{50}, half-maximal inhibitory concentration; **TOP1**, topoisomerase I.

FIGURE 5. HWK-206 Shows Greater Inhibition of Cell Viability Than ABBV-706 in Cell Lines with Varying Levels of SEZ6 Expression



Cell line	HWK-206	ABBV-706	ABBV-706-mAb-CPT113	Isotype-CPT113
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)
NCI-H69 SEZ6^{low}	0.47	36	6.4	>100
NCI-H524 SEZ6^{medium}	0.22	1.6	4.5	>100
SHP77.hSEZ6^{high}	0.01	0.02	0.02	>100

Cell lines with varying levels of SEZ6 expression—NCI-H69 SEZ6^{low}, NCI-H524 SEZ6^{medium}, and SHP77.hSEZ6^{high}—were incubated with HWK-206, ABBV-706, isotype-CPT113 (isotype-matched control mAb conjugated to CPT113), or ABBV-706-mAb-CPT113 (ABBV-706 mAb conjugated to CPT113) at concentrations of 0.015–100 nM for 7 days at 37°C. Cell viability was assessed using the Cell Titer-Glo luminescent assay (Promega).

IC_{50}, half-maximal inhibitory concentration; **mAb**, monoclonal antibody; **SEZ6**, seizure-related homolog protein 6.

IN VIVO TUMOR ACTIVITY

» HWK-206 showed robust antitumor activity in an SCLC model, with tumor growth inhibition ranging from 90% to 104% depending on the HWK-206 dose (**Figure 6**)

– Tumor growth remained suppressed up to 21 days after HWK-206 administration and resulted in regressions at doses as low as 2 mg/kg

» HWK-206 showed greater antitumor activity than ABBV-706 mAb conjugated to CPT113 (**Figure 7**), highlighting the increased activity of the HWK-206 bpAb

» HWK-206 demonstrated potent antitumor activity comparable to ABBV-706 (**Figure 7**)

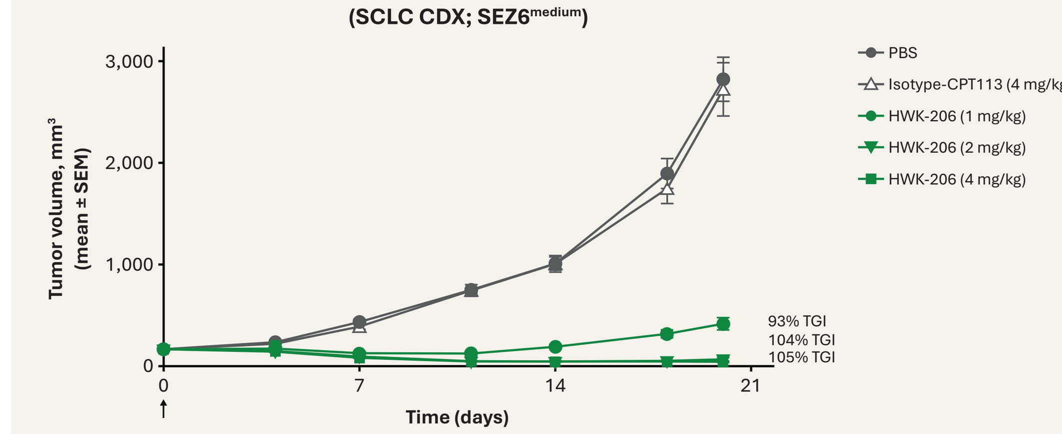
PHARMACOKINETICS

» HWK-206 was stable in human plasma with minimal (<0.02%) free CPT116 payload detected after 14 days of incubation at 37°C (data not shown)

» Plasma levels of HWK-206 ADC were stable with a half-life of 7–9 days in cynomolgus monkeys treated with HWK-206. Very low levels of free CPT116 payload (0.01%) were detected (**Figure 8**)

» The highest non-severely toxic dose (HNSTD) of HWK-206 was considered to be 60 mg/kg; this was the highest dose tested in a study in which cynomolgus monkeys were administered intravenous HWK-206 once every 3 weeks for 22 days (two doses in total)

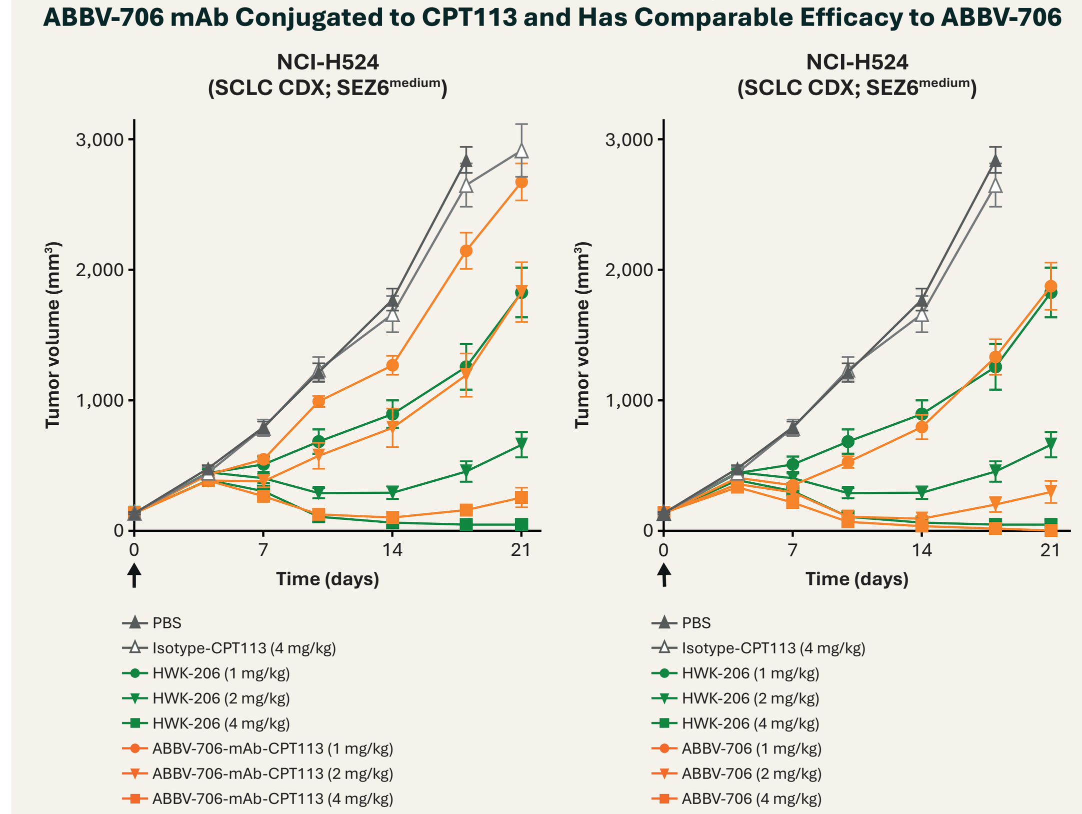
FIGURE 6. HWK-206 Biparatopic ADC Shows Robust Antitumor Activity in an SCLC Model



Antitumor activity of HWK-206 was evaluated in an SCLC CDX model. A single intravenous dose of HWK-206, isotype-CPT113 (isotype-matched control mAb conjugated to CPT113), or PBS was administered to eight mice in each group after tumors had grown to 150 mm^3 (day 0), as indicated by the arrow. Percentage TGI was calculated as TGI (%) = $[1 – (T_i – T_0) / (V_i – V_0)] \times 100\%$, in which T_i = volume of treatment group on day i after administration; T_0 = mean tumor volume of treatment group on day 0 after administration; V_i = 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.

CDX, cell line-derived xenograft; **mAb**, monoclonal antibody; **PBS**, phosphate-buffered saline; **SCLC**, small cell lung cancer; **SEZ6**, seizure-related homolog protein 6; **TGI**, tumor growth inhibition.

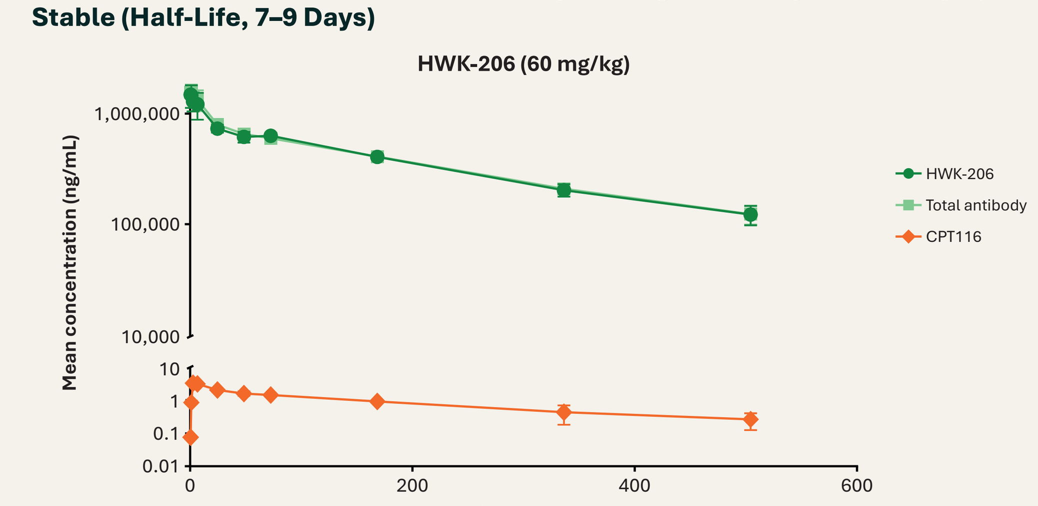
FIGURE 7. HWK-206 Demonstrates Antitumor Activity Greater than ABBV-706 mAb Conjugated to CPT113 and Has Comparable Efficacy to ABBV-706



Antitumor activity of HWK-206 compared with ABBV-706-mAb-CPT113 (ABBV-706 mAb conjugated to CPT113) and ABBV-706 ADC was evaluated in an SCLC CDX model. A single intravenous dose of HWK-206, isotype-CPT113 (isotype-matched control mAb conjugated to CPT113), ABBV-706-mAb-CPT113, ABBV-706, or PBS was administered to eight mice in each group after tumors had grown to 150 mm^3 (day 0), as indicated by the arrow. Percentage TGI was calculated as TGI (%) = $[1 – (T_i – T_0) / (V_i – V_0)] \times 100\%$, in which T_i = volume of treatment group on day i after administration; T_0 = mean tumor volume of treatment group on day 0 after administration; V_i = 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.

ADC, antibody-drug conjugate; **CDX**, cell line-derived xenograft; **mAb**, monoclonal antibody; **PBS**, phosphate-buffered saline; **SCLC**, small cell lung cancer; **SEZ6**, seizure-related homolog protein 6.

FIGURE 8. HWK-206 Is Well Tolerated in Cynomolgus Monkeys and Is Highly Stable (Half-Life, 7–9 Days)



HWK-206, total antibody, or free payload (CPT116) was administered as a single dose. Plasma was collected from time 0 to 504 h and purified. HWK-206 and antibody concentrations were assessed using an enzyme-linked immunosorbent assay. CPT116 was quantified using triple quadrupole liquid chromatography with tandem mass spectrometry.

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