HP004, a Novel Antibody-Drug Conjugate Targeting Alkaline Phosphatases ALPP and ALPPL2 in Solid Tumors

#6776

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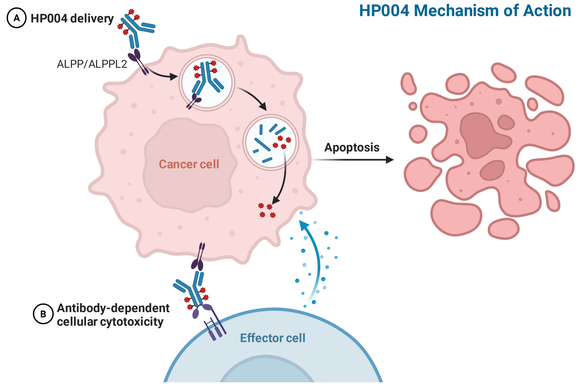
Abstract

Alkaline phosphatase placental (ALPP) and ALP-like 2 (ALPPL2) are highly expressed in a wide set of solid tumors, including ovarian, endometrial, gastric and non-small cell lung cancers. Their expression is highly restricted in normal tissues. ALPP/ALPPL2 are attractive targets for antibody-drug conjugate (ADC) and CAR-T therapies.

HP-004 is a novel ADC targeting both ALPP and ALPPL2, with high selectivity and enhanced internalization efficiency. HP-004 specifically binds ALPP/ALPPL2 but not the related isozymes ALPI or ALPL, minimizing potential on-target off-tumor liability. In cellular internalization assays using ALPP/ALPPL2 high- and low-expressing tumor cells, HP-004 demonstrated higher uptake and an improved capacity to deliver cytotoxic payload intracellularly across a range of target expression levels. HP-004 is site-specifically conjugated with monomethyl auristatin E (MMAE) with an average DAR about 4. In vitro, HP-004 induced potent target-dependent cytotoxicity in ALPP/ALPPL2-overexpressing tumor cell lines, with an IC_{50} of approximately 248 pM. In the ALPP/ALPPL2-positive NCI-H1651 xenograft lung cancer model, HP-004 administration achieved near-complete tumor regression at 3 mg/kg (BiW X2), with complete tumor regression in 6 of 8 mice, demonstrating a robust antitumor activity. HP-004 is further being evaluated in cell line- and patient-derived xenograft models with different expression levels of ALPP and ALPPL2. In parallel, bispecific ADC formats incorporating HP-004 are being explored to further enhance therapeutic index and extend activity to heterogeneous or low-antigen tumors. Collectively, our data support HP-004 as a highly selective ADC candidate with superior internalization, strong preclinical efficacy, and promising potential for translation in solid tumors expressing ALPP/ALPPL2.

HP004 Mechanism of Action



- HP004 is a human IgG1 antibody–drug conjugate (ADC) directed against ALPP/ALPPL2 that exerts its antitumor activity through both payload-mediated cytotoxicity and Fc-dependent effector functions.
- After specific binding to ALPP/ALPPL2 on the surface of tumor cells, HP004 is internalized and trafficked to the endo-lysosomal compartment, where the linker is cleaved and the cytotoxic payload is released to induce intracellular damage (e.g., cell-cycle arrest and apoptosis), leading to potent, target-dependent tumor cell killing and potential bystander effects in antigen-low neighboring tumor cells.
- In parallel, the intact IgG1 Fc region engages Fcγ receptors on NK cells and other effector cells, triggering antibody-dependent cell-mediated cytotoxicity (ADCC) and further amplifying tumor cell clearance beyond the direct ADC effect.
- Together, these mechanisms provide dual killing via targeted payload delivery and immune-mediated cytotoxicity, aiming to achieve deep and durable tumor burden reduction in ALPP/ALPPL2-positive tumors.

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ALPP/ALPPL2 Expression in Cancer

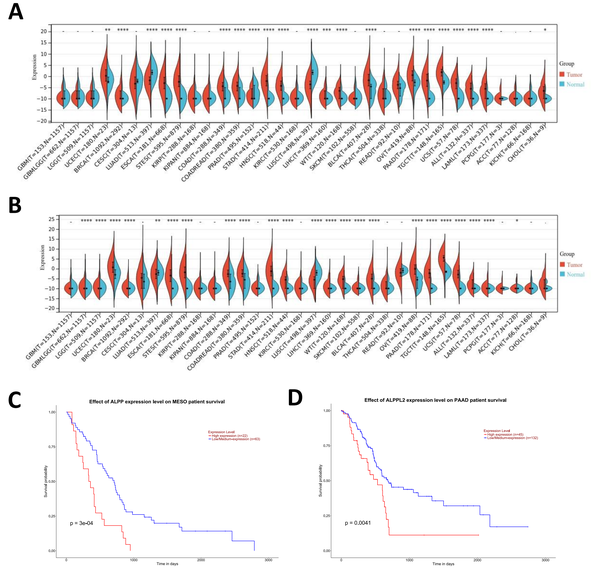


Figure 1: ALPP and ALPPL2 expression across tumor types and association with patient survival. **(A–B)** Differential expression of ALPP **(A)** and ALPPL2 **(B)** across multiple cancer types based on TCGA datasets, comparing tumor (red) versus normal (blue) tissues. Both ALPP and ALPPL2 show elevated expression in multiple tumor types relative to normal tissues. **(C–D)** Kaplan-Meier survival analyses demonstrates the association between gene expression and overall survival. High ALPP expression is associated with significantly poorer survival in mesothelioma (MESO) patients (p = 3e-04), while elevated ALPPL2 expression correlates with reduced survival in pancreatic adenocarcinoma (PAAD) patients (p = 0.0041).

Anti-ALPP/ALPP2 Antibody Characteration for Binding, Internalization and Cytotoxicity

A

	NCI-H1651	NUGC-3
Receptor (×10³)	500	191
ALPP mRNA (TPM+1)	165	58
ALPPL2 mRNA (TPM+1)	665	11

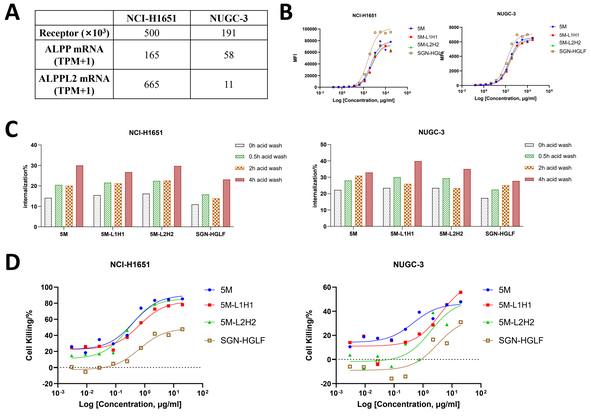
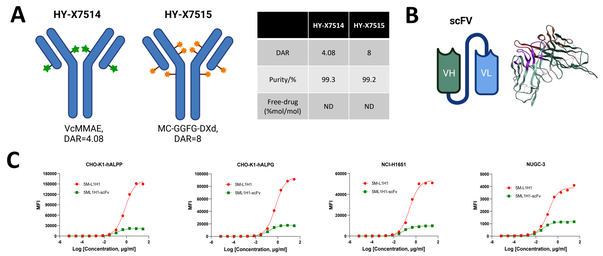


Figure 2: Comparison of antibody binding, internalization, and cytotoxic activity with SEAGEN reference antibody. **A.** Summary of ALPP, ALPPL2 expression on NCI-H1651 and NUGC-3 tumor cell lines. **B.** Binding activity of indicated antibodies measured by flow cytometry. Representative dose-response curves show mean fluorescence intensity (MFI) as a function of antibody concentration.. **C.** Quantification of antibody internalization across different ALPP/ALPPL2 overexpressing tumor cells. Antibody internalization is presented as the percentage of internalized fraction under acid-wash conditions. **D.** In vitro cytotoxicity of antibodies evaluated using DT3C-mediated killing assays, with cell viability measured by CCK-8. Dose-response curves are shown as the percentage of cell killing versus log-transformed antibody concentration in two tumor cell models.

Antibody Engineering for ADC and RDC



	HY-X7514	HY-X7515
DAR	4.08	8
Purity/%	99.3	99.2
Free-drug (%mol/mol)	ND	ND

Figure 3: Characterization of HP004 antibody formats and binding properties. **A**. Schematic illustration of HP004 ADC constructs with different drug-to-antibody ratios (DAR). Conjugation profiles for HY-X7514 and HY-X7515 are shown, with corresponding DAR, purity, and free-drug levels summarized. **B.** Structural model of the HP004 scFv (VH–VL) generated by AlphaFold prediction, highlighting the overall folding and variable domain organization. **C.** Binding activity of the scFv format compared with the parental antibody, measured by flow cytometry. Representative dose–response curves (MFI vs. log concentration) are shown across engineered cell lines (CHO-K1-hALPP, CHO-K1-hALPPL2) and tumor cell lines (NCI-H1651, NUGC-3). The scFv retained target binding ability but exhibited reduced binding strength compared with the full-length antibody across all tested cell models.

HP004 ADC Binding to ALPP and ALPPL2 on Tumor Cells

A

Antibody	CHO-K1-hALPP	CHO-K1-hALPPL2	NCI-H1651	NUGC-3
5M-L1H1	0.838	0.721	0.398	0.382
HY-X7515	0.082	0.135	0.132	0.133
HY-X7514	1.152	0.570	0.434	0.405

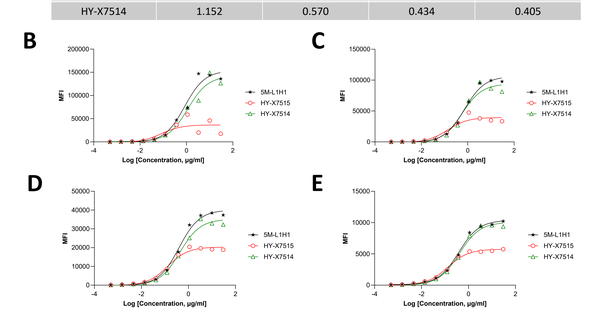


Figure 3: Binding of HP004 ADC to ALPP and ALPPL2 on tumor cells. A. Summary of binding (EC50, ug/ml) of indicated antibodies to ALPP- and ALPPL2-expressing cells, including engineered CHO-K1-hALPP and CHO-K1-hALPPL2 cells, as well as tumor cell lines NCI-H1651 and NUGC-3. **(B-E)** Representative flow cytometry–based binding curves showing concentration-dependent binding of antibodies (5M-L1H1, HY-X7515, HY-X7514) to target-expressing cells. Data are presented as mean fluorescence intensity (MFI) versus log-transformed antibody concentration. **(B)** Binding to CHO-K1-hALPP cells. **(C)** Binding to CHO-K1-hALPPL2 cells. **(D)** Binding to NCI-H1651 cells. **(E)** Binding to NUGC-3 cells.

HP004 ADC Internalization into Tumor Cells Expressing ALPP and ALPPL2

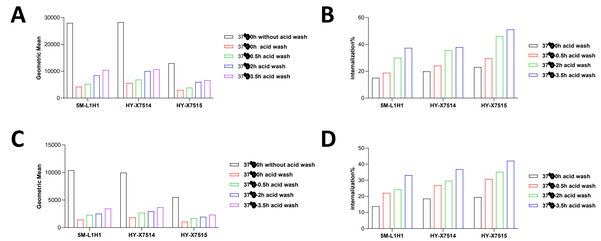


Figure 4: Efficient internalization of HP004 ADC in ALPP/ALPPL2-expressing tumor cells. **(A, C)** Quantification of cell-associated antibody signal (geometric mean fluorescence intensity, MFI) following incubation at 37 °C with or without acid wash. Acid wash removes surface-bound antibodies, allowing discrimination of internalized fractions over time (0, 0.5, 2, and 3.5 h). **(B, D)** Corresponding internalization percentages calculated from acid-wash conditions, demonstrating time-dependent increases in intracellular accumulation. **(A-B)** Internalization in NCI-H1651 tumor cells. **(C–D)** Internalization in NUGC-3 tumor cells.

HP004 *in vitro* Effectively Kills Tumor Cells Expressing ALPP/ALPPL2

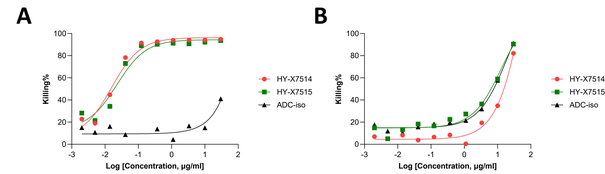


Figure 5: HP004 ADC–mediated cytotoxicity against ALPP- and ALPPL2-expressing tumor cells in vitro. In vitro cytotoxicity of HP004 ADC evaluated by CCK-8 assay following treatment with increasing concentrations of indicated antibodies (HY-X7514, HY-X7515) or isotype control ADC (ADC-iso). Cell killing is presented as the percentage of growth inhibition versus log-transformed antibody concentration. **(A)** Cytotoxic activity in NCI-H1651 tumor cells. **(B)** Cytotoxic activity in NUGC-3 tumor cells. HP004 ADC demonstrated potent and dose-dependent tumor cell killing in both models, whereas minimal cytotoxicity was observed with the isotype control ADC.

HP004 Bystander Killing Activity

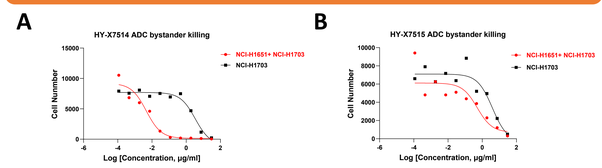


Figure 6: Bystander killing activity of HP004 ADC in co-culture tumor model. **A.** Bystander killing activity of HY-X7514 ADC (MMAE, DAR = 4.08) evaluated in a co-culture system of ALPP/ALPPL2-positive NCI-H1651 cells and antigen-negative NCI-H1703 cells. Cell viability was assessed following treatment with increasing ADC concentrations, and cell numbers are plotted against log-transformed concentration. HY-X7514 induced potent killing in the mixed-cell population (NCI-H1651 + NCI-H1703), while showing limited activity in NCI-H1703 monoculture, indicating a strong bystander effect. **B.** Bystander killing activity of HY-X7515 ADC (DXd, DAR = 8) assessed under the same conditions. Compared with HY-X7514, HY-X7515 demonstrated weaker bystander killing, with reduced potency in the co-culture model.

Robust *in vivo* Antitumor Efficacy of HP004

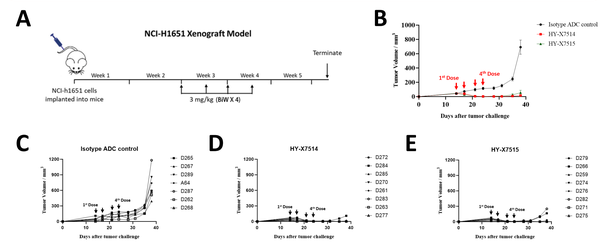


Figure 7: Robust in vivo antitumor efficacy of HP004**. A.** Schematic representation of the *in vivo efficacy* study design. Tumor-bearing mice were treated with HP004 via repeated dosing as indicated (arrows). Tumor growth was monitored over time and compared with control treatment. **B.** Tumor growth kinetics following HP004 treatment. Arrows indicate dosing time points. HP004 treatment resulted in sustained tumor growth inhibition compared with the control, with divergence observed following repeated dosing. **(C-E)** Tumor growth curves for individual mice in different treatment groups, demonstrating consistent and reproducible tumor suppression across treated animals compared with the control ADC.

Summary

- HP004 are human IgG1 antibody–drug conjugates (ADCs) directed against ALPP/ALPPL2 that exert antitumor activity through both payload-mediated cytotoxicity and Fc-dependent effector functions.
- HP004's anti-ALPP/ALPPL2 antibody has improved internalization efficiency and cytotoxic activity compared with SGN reference antibody.
- HY-X7514 is conjugated with MMAE with an average DAR about 4. HY-X7515 is conjugated with DXd with an average DAR about 8. Both ADC candidates demonstrated potent target-dependent cytotoxicity *in vitro* and consistent anti-tumor efficacy *in vivo*. Both HY-X7514 and HY-X7515 led to sustained tumor growth inhibition in xenograft tumor models.