Discovery of HMPL-A251, a first-in-class HER2-directed antibody-targeted therapy conjugate (ATTC) with a novel PI3K/PIKK inhibitor payload

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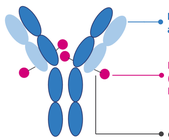
INTRODUCTION

- Although toxin-based ADCs have revolutionized cancer therapy, their long-term use remains hindered by severe toxicities that mirror those of their cytotoxic payloads. These safety challenges further limit their combination potential with chemotherapy, highlighting the need for innovative payload design[1].
- Aberrant activation of PI3K-AKT-mTOR pathway (PAM) is associated with poor prognosis and resistance to anti-HER2 therapies[2-3]. Despite the synergistic effects of dual HER2 and PAM inhibition, systemic toxicity associated with PAM inhibitors limits their clinical application[4], providing the rationale for developing HMPL-A251, a novel and potent PI3K/PIKK inhibitor conjugated with a HER2-targeting antibody.



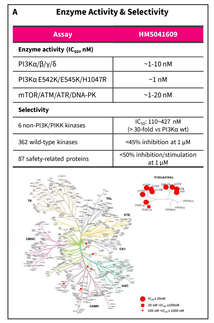
- HER2 overexpression occurs in a variety of solid tumors[5]
- HER2 overexpression is associated with poor prognosis[6-9], increased risk of disease recurrence[9], and resistance to anti-cancer treatment[10]

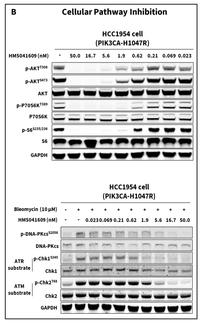
- Highly potent against PI3K and PIKK kinases
- Synergizes with anti-HER2 antibody to improve efficacy
- PIKK inhibition enables the potential of chemotherapy combinations
- Bystander effect to kill antigen negative tumor cells

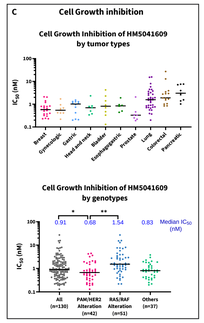
- Stable in human and monkey plasma
- Cleaved by cathepsin B, a protease highly expressed in cancer cells

RESULTS

In vitro profile of the payload HM5041609



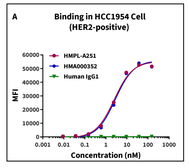


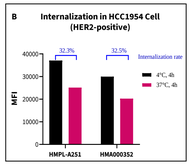


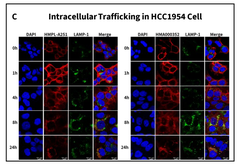
A, Enzyme activity against PI3K and PIKK kinases and selectivity. The inhibitory activity of HM5041609 towards PI3K and PIKK kinases were determined using radiometric and HTRF assays by Eurofins; the selectivity of HM5041609 was evaluated against a panel of 418 kinases (KinaseProfiler™) and 87 safety-related proteins (SafetyScreen87™) at 1 μM by Eurofins. **B, Inhibition of PAM and PIKK pathways in HCC1954 cells.** Cells were treated with HM5041609 at indicated concentrations for 2 hours and lysed for western blot assay to assess the modulation on PAM pathway; to evaluate the impact on PIKK signaling, cells were pre-incubated with HM5041609 for 1 hour, followed by bleomycin treatment for 6 hours. **C, Cell growth inhibitory activity.** 130 tumor cell lines were treated with HM5041609 for 6 days, and cell viability was assessed using CCK-8 or luminescent 3D cell viability assays. PAM alterations include oncogenic or likely oncogenic mutation of PIK3CA/PIK3CB/PIK3R1/PTEN, or PTEN decrease/loss by immunohistochemistry staining; HER2 alterations include HER2 CN>10, oncogenic or likely oncogenic mutation of HER2. RAS/RAF alterations include KRAS/NRAS/HRAS/BRAF/RIT1 oncogenic or likely oncogenic mutations or KRAS CN>10. The gene alteration information of tumor cell lines was obtained from CCLE and OncoKB® database or from in-house profiling data. Statistical analysis was performed with Mann-Whitney test. *: p<0.05, **: p<0.01.

- **HM5041609 is a highly potent and selective inhibitor of PI3K and PIKK kinases with robust anti-tumor activity against a broad panel of tumor cell lines.**

Binding and internalization of HMPL-A251





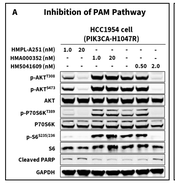


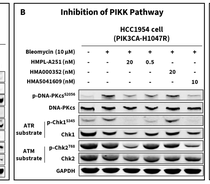
A, Cell surface HER2 protein binding. The binding affinity of naked antibody or HMPL-A251 to cell surface HER2 protein in HER2-positive human breast cancer HCC1954 cells was assessed by flow cytometry. **B, Internalization rate of HMPL-A251.** HCC1954 cells were pretreated with naked antibody or HMPL-A251 at 4℃ for 1 hour, washed, and further incubated at 37℃ or 4 ℃ for 4 hours. Cell surface retention of PE-conjugated anti-human IgG Fc-bound antibodies in control (4℃) versus internalization (37 ℃) groups was quantified by flow cytometry. The internalization rate (%) was calculated using the following equation:(4℃ MFI-37℃ MFI)/4℃ MFI×100%. **C, Intracellular trafficking of HPML-A251 to lysosome.** HCC1954 cells were pretreated with naked antibody or HMPL-A251 at 4℃ for 30 minutes, washed, and incubated at 37℃. Time-dependent colocalization analysis of the antibody/HMPL-A251 (red), lysosomal marker LAMP-1 (green), and nuclear DAPI (blue) was performed via confocal microscopy.

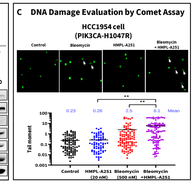
- **HMPL-A251 displayed high binding affinity and efficient internalization to HER2-positive breast cancer HCC1954 cells, comparable to the naked antibody.**

RESULTS

Cellular signaling pathway inhibition of HMPL-A251



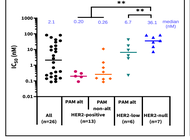


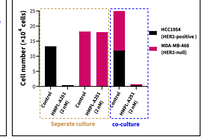


A, Inhibition of PAM pathway in HCC1954 cells. Cells were treated with compounds at indicated concentrations for 16 hours and lysed for Western blot assay to assess the modulation on PAM pathway. **B, Inhibition of PIKK pathways in HCC1954 cells.** Cells were pre-incubated with HMPL-A251/naked antibody for 8 hours or HM5041609 for 1 hour, followed by bleomycin treatment for 6 hours and lysed for PIKK pathway evaluation by Western blot assay. **C, DNA damage evaluation.** Cells were pre-incubated with HMPL-A251 or control for 8 hours followed by bleomycin or control treatment for 9 hours and subjected to alkaline comet assay to detect DNA damage. The damaged DNA was quantified by tail moment (Tail DNA% x Tail Length). Statistical analysis was performed with one-way ANOVA test. *: p<0.05, **: p<0.01.

- **HMPL-A251 potently blocked intracellular PAM (PI3K/AKT/mTOR) and PIKK (ATM/ATR/DNA-PK) signaling pathways, leading to subsequent induction of apoptosis and DNA damage.**

Cell growth inhibition of HMPL-A251

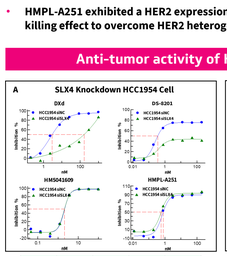




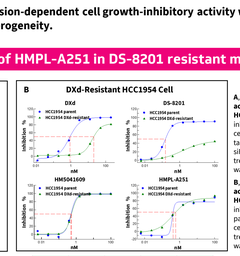
A, Cell growth inhibitory activity of HMPL-A251. 26 tumor cell lines with varying HER2 expression levels were treated with HMPL-A251 for 6 days, and cell viability was assessed using CCK-8 or luminescent 3D cell viability assays. HER2-positive: IHC3+ or IHC2+/FISH+; HER2-low: IHC2+/FISH- or IHC1+; HER2-null: IHC 0; PAM alteration (alt): oncogenic or likely oncogenic mutations in PIK3CA/PTEN, and PTEN loss by IHC. Statistical analysis was performed with Student's t test. *:p<0.05, **: p<0.01. **B, Bystander killing effect of HMPL-A251.** HER2-positive HCC1954 cells and HER2-null MDA-MB-468 cells were evaluated in both separate and co-culture (2:1 ratio) conditions and treated with 2 nM HMPL-A251 for 6 days. Cell number was counted by flow cytometry.

- **HMPL-A251 exhibited a HER2 expression-dependent cell growth-inhibitory activity with bystander killing effect to overcome HER2 heterogeneity.**

Anti-tumor activity of HMPL-A251 in DS-8201 resistant model



	DXd	DS-8201	HM5041609	HMPL-A251
IC_{50} shift	55.3x	>556x	1.0x	0.75x



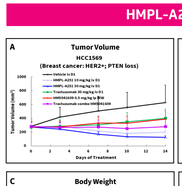
	DXd	DS-8201	HM5041609	HMPL-A251
IC_{50} shift	23.2x	>667x	1.1x	1.4x

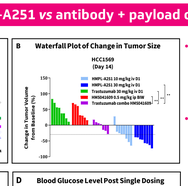
A, Cell growth inhibitory activity in SLX knockdown HCC1954 cells. Dose-response inhibition curves of HCC1954 cells transfected with non-targeting or SLX4-targeted siRNAs, and exposed to indicated treatment for 5 days, cell viability was assessed using CCK-8 assay.

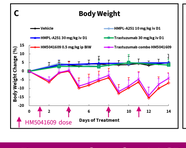
B, Cell growth inhibitory activity in DXD-resistant HCC1954 cells. Dose-response inhibition curves of HCC1954 parent and DXd-induced resistant cells exposed to indicated treatment for 6 days, cell viability was assessed using CCK-8 assay.

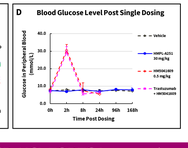
- **HMPL-A251 can overcome the DXd-mediated resistance to DS-8201.**

HMPL-A251 *vs* antibody + payload combination





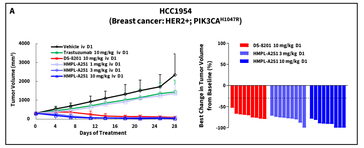


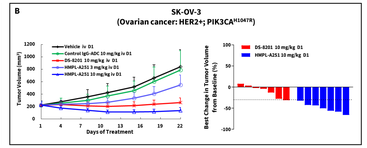


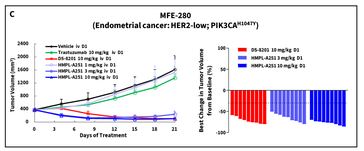
- **Combination of HM5041609 and trastuzumab produced synergistic anti-tumor effect but caused safety issue as revealed by body weight loss and increase in blood glucose.**
- **HMPL-A251 demonstrated stronger anti-tumor activity and better tolerability than antibody and payload combination.**

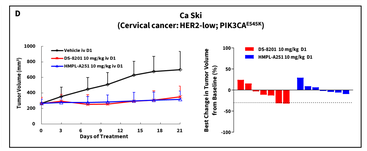
A-C, *In vivo* anti-tumor effect in HCC1569 subcutaneous xenograft model. HCC1569 tumor-bearing CB-17 SCID mice were treated with HMPL-A251 (10 or 30 mg/kg, iv, day1), HM5041609 (0.5 mg/kg, ip, twice a week), trastuzumab (30 mg/kg, iv, day 1), or HM5041609 in combination with trastuzumab. Each point in **A** and **C** represents mean and SD (n=8). **D, Glucose levels in peripheral blood.** Glucose levels in peripheral blood were measured at indicated time points after the first dose of treatment. Each point represents mean and SD (n=4). Statistical analysis was performed with Student's t test. *:p<0.05, **: p<0.01. iv: intravenous; ip: intraperitoneal; D1: day 1; BIW: twice a week.

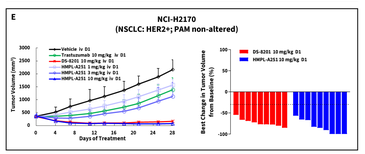
In vivo anti-tumor efficacy of HMPL-A251

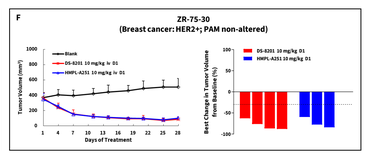








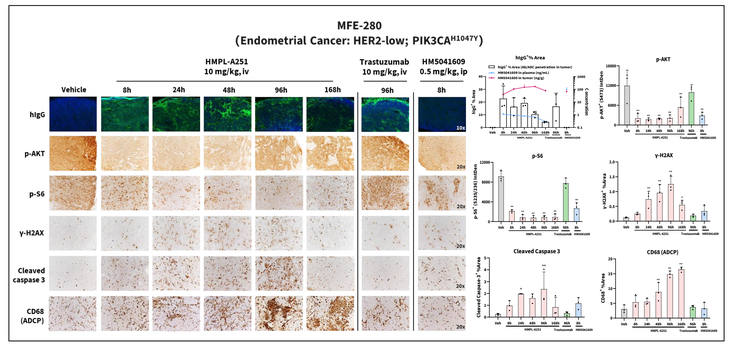




Immuno-deficient mice bearing indicated tumor xenografts, including HER2+/PAM altered (**A-B**), HER2-low/PAM altered (**C-D**), and HER2+/PAM-non-altered (**E-F**), were treated with a single intravenous dose of DS-8201 or HMPL-A251 on day 1. Trastuzumab or control IgG-ADC with same linker-payload were used as control in some models. Tumor volume was measured twice a week to assess the anti-tumor activity. Each point represents mean and SD. Best change in tumor volume from baseline refers to the maximum percentage of tumor shrinkage from baseline in the compound treatment group, which was illustrated by the change in tumor volume of individual animal at the day of the best tumor regression achieved. NSCLC: non-small cell lung cancer; iv: intravenous; D1: day 1; alt: alteration. Dash line means 30% reduction of tumor volume from baseline.

- **A single intravenous dose of HMPL-A251 demonstrated robust anti-tumor activity in HER2-positive tumor models with or without PAM alterations as well as HER2-low tumor models harboring PAM alterations, which was comparable or stronger than DS-8201 administered at an equivalent dose.**

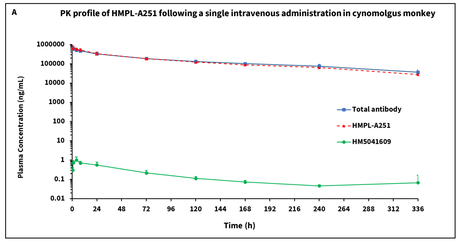
In vivo pharmacodynamic activity of HMPL-A251

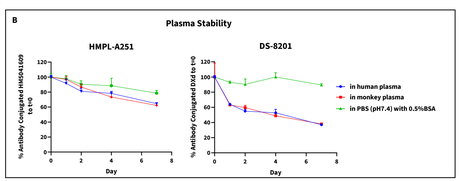


MEF-280 tumor-bearing NOD-SCID mice were treated with a single dose of HMPL-A251 (10 mg/kg, iv), trastuzumab (10 mg/kg, iv) or HM5041609 (0.5 mg/kg, ip) and euthanized at different time points. Tumor tissues were collected and analyzed for different biomarkers by immunofluorescence or immunohistochemistry staining method. hIgG immunofluorescence staining was used to determine tumor distribution of HMPL-A251. Meanwhile, concentrations of unconjugated HM5041609 in plasma and tumor tissue were determined by LC-MS/MS. Data were expressed as mean and SD (n=3). Differences between treatment and vehicle group were compared using one-way ANOVA. *: p<0.05, **: p<0.01. iv: intravenous; ip: intraperitoneal; IntDen: integrated density.

- **HMPL-A251 quickly distributed into the tumor and persisted for 168 hours following a single dose, enabling sustained intra-tumoral payload release with subsequent potent and durable inhibition of PI3K pathway as well as remarkable induction of DNA damage and tumor cell apoptosis.**
- **A significant accumulation of CD68-positive macrophages was observed from 48 hours and continued to rise up to 168 hours, suggesting strong antibody-dependent cell-mediated phagocytosis (ADCP) function by HMPL-A251.**
- **Compared with systemic administration of HM5041609, HMPL-A251 treatment achieved a superior tumor-to-plasma ratio, thereby potentially minimizing payload-related systemic toxicity while maintaining therapeutic efficacy.**

Pharmacokinetic profile and GLP monkey toxicity study





A, Pharmacokinetics of HMPL-A251 in cynomolgus monkeys. HMPL-A251 was intravenously administered to cynomolgus monkeys at the dose of 30 mg/kg and the concentrations of HMPL-A251, total antibody and HM5041609 in plasma were determined. Each value represents the mean and SD (n=10, 5 females and 5 males). **B, *In vitro* stability of HMPL-A251 and DS-8201 in plasma.** 100 μg/mL HMPL-A251 and DS-8201 were incubated in the control buffer (PBS pH7.4 with 0.5% BSA), monkey or human plasma at 37℃ for up to 7 days. The remaining conjugated payload at each timepoint was determined and normalized to day 0. Each value represents the mean and SD (n=3).

- **Following a single intravenous administration in cynomolgus monkeys, HMPL-A251 demonstrated a favorable PK profile with low clearance. The similar PK profile between HMPL-A251 and total antibody indicated good linker stability during circulation.**
- **The plasma exposure of unconjugated HM5041609 was much lower than total antibody and HMPL-A251 (molar ratio:<0.5%), indicating low risk of payload-based toxicities.**
- **HMPL-A251 was more stable than DS-8201 in human and monkey plasma.**
- **The 29-day GLP toxicology study in cynomolgus monkeys shows no on-target, off-tumor hyperglycemia or myelosuppression, indicating a well-tolerated safety profile for HMPL-A251.**
- **The ATTC approach effectively mitigates PI3K/PIKK toxicities, supporting further clinical development of HMPL-A251.**

SUMMARY

- **HMPL-A251, a first-in-class PI3K/PIKK-HER2 antibody-targeted therapy conjugate, has demonstrated potent anti-tumor activity in HER2-positive tumor models, with or without PAM alterations, as well as HER2-low tumor models harboring PAM alterations.**
- **HMPL-A251 exhibited favorable pharmacokinetic and safety profiles, warranting further clinical evaluation.**
- **US IND was cleared. China IND is under review. Phase 1 clinical study is expected to start in Q4 2025.**

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