AACR American Association for Cancer Research®

ANNUAL MEETING 2023

APRIL 14-19 • #AACR23

Pre-clinical evaluation of a novel antibody drug conjugate (ADC) LM-306 targeting IL-13Rα2 in immuno-oncology

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Abstract Presentation Number: **1881**

Introduction

- Interleukin-13 receptor subunit alpha-2 (IL-13Rα2) is a high-affinity membrane receptor of IL-13 abundantly expressed in wide range of tumours.[1-3]
- IL-13Rα2 is over-expressed in 83% of pediatric and 58% of adult brain tumours,38.7% in glioma tissues and 7.5% in melanomas.[1, 3]
- Overexpression of IL-13Rα2 also increases the invasiveness and metastatic potential of cancer cells and hence could be a potential target for anti-cancer therapy.[1]
- Several studies have substantiated the promising role of IL-13Rα2 against gliomas in immunotherapy trials.[1]
- LM-306, a novel antibody-drug conjugation (ADC) developed to target IL-13Rα2, is comprised of a recombinant humanized anti-IL-13Rα2 IgG1 mAb (LM-106) coupled with cytotoxic payload MMAE.
- Preclinical studies were conducted to characterize LM-306 in IL-13Rα2-positive cells, including binding affinity, internalization, Antibody-dependent cell-mediated cytotoxicity (ADCC), cell cytotoxicity, bystander effect and *in vivo* efficacy in melanoma models.

LM-306 Mechanism of Action

- *Linker cleavable*
- *Payload antimitotic agent: vc-MMAE*

Methods

In vitro assays

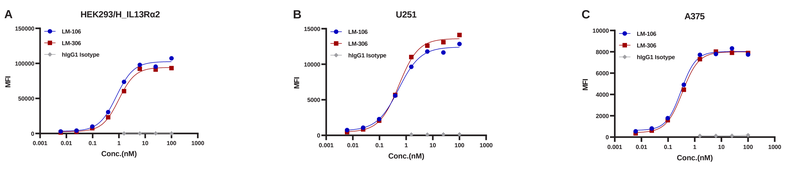
- Antibody binding activity to Human IL-13Rα2 expressing cells and endogenous IL-13Rα2 expressing cancer cells were tested by flow cytometry.
- Cross-species reactivity of antibody was assessed in rhesus, cynomolgus, mouse and rat IL-13Rα2 overexpressing cell lines and antibody binding affinity were assessed in Bio-Layer interferometry assay.
- The internalization efficacy of antibodies toward human IL-13Rα2 expressing cell lines were determined using fluorescence intensity.
- ADCC direct cytotoxicity and bystander effect were assessed using viability assays.

In vivo assays

- Anti-tumour activity was investigated using A375 CDX model.

Results

LM-306 Potently Bind to Human IL-13Rα2 Expressing Cell and Endogenous IL-13Rα2 Expressing Cancer Cells



Cell Line / EC_{50}(nM)	HEK293/H_IL-13Rα2	U251	A375
LM-106	0.79	0.53	0.31
LM-306	0.99	0.53	0.35

Figure 1: **Cellular Binding.**

Cell based binding of antibodies to Human IL-13Rα2 expressing cell line were assessed using flow cytometry. LM-106 and LM-306 potently bind to human IL-13Rα2-expressing cells **(A),** endogenous IL-13Rα2 expressing U251 Glioma cancer cells **(B)** and A375 Melanoma cancer cells **(C)** in a dose-dependent manner.

LM-106 Cross-Reacted with Rhesus and Cynomolgus Monkey IL-13Rα2

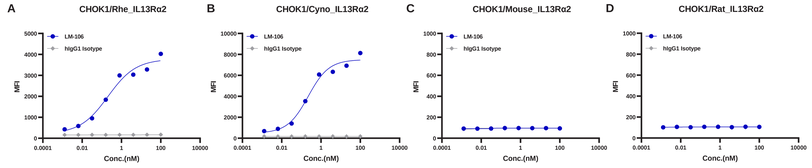


Figure 2: **Cross-species Binding of LM-106.**

CHOK1 cells overexpressing Rhesus **(A)** cynomolgus monkey **(B)** Mouse **(C)** and rat **(D)** IL-13Rα2 were incubated in the presence of a range of concentrations of LM-106, the naked antibody of LM-306. LM-106 only can effectively bind to rhesus, cynomolgus monkey IL-13Rα2 in a dose dependent manner with EC_{50} of 0.21nM and 0.22nM, respectively.

Binding Affinity Test by Bio-Layer interferometry assay

Antibody / Antigen	LM-106 KD (M)	LM-106 ka (1/Ms)	LM-106 kd (1/s)	LM-106 Rmax (nm)	LM-306 KD (M)	LM-306 ka (1/Ms)	LM-306 kd (1/s)	LM-306 Rmax (nm)
human IL13Rα2	1.77E-09	2.79E+05	4.93E-04	0.9905	7.43E-10	2.37E+05	1.76E-04	0.9766
Rhesus_IL13Rα2	7.56E-10	155213.4	1.17E-04	1.3066	4.84E-10	142208.1	6.88E-05	1.3313

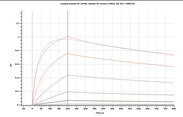
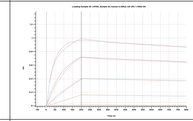
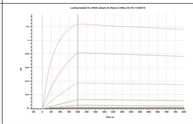
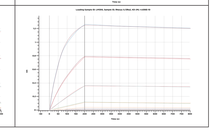
Antigen	LM-106	LM-306
Human IL13Rα2		
Rhesus_IL13Rα2		

Figure 3: **Binding Affinity of Antibodies to Human and Rhesus Monkey IL-13Rα2 Test by Bio-Layer interferometry assay.**

Test antibodies were diluted to 5μg/mL and immobilized to Protein A Dip and Read Biosensors. 3-folds serial dilutions of Human IL-13Rα2 and Rhesus IL-13Rα2 starting from 100nM and used as analytes. The equilibrium dissociation constant(KD) was calculated as the ratio kd/ka. Data were analyzed and KD was calculated by software Octet Analysis Studio 12.2. Binding affinity of LM-306 to human and rhesus monkey IL-13Rα2 were comparable with LM-106 (KD:7.43E-10 vs 1.77E-9, KD: 4.84E-10 vs 7.56E-10).

LM-106 Internalized in Human IL-13Rα2 Expressing Cell and Cancer Cells

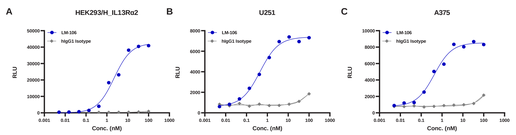


Figure 4: **Internalization of LM-106.**

Antibodies were conjugated with pHAb dye, which is not fluorescent at neutral pH but becomes highly fluorescent at acidic pH in lysosome with internalization. IL-13Rα2-positive cells were incubated with Antibody-pHdye conjucation for 24 hours and detected fluorescence by Envison. LM-106 showed concentration-dependent internalization in human IL-13Rα2-expressing cells **(A)**, endogenous IL-13Rα2 expressing U251 Glioma cancer cells **(B)** and A375 Melanoma cancer cells **(C)** with EC_{50} of 2.23nM, 0.44nM and 0.43nM, respectively.

LM-106 Showed Strong Concentration-Dependent ADCC in IL-13Rα2-Positive cells

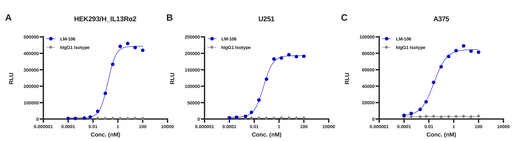


Figure 5: **LM-106 Induces Potent ADCC Effects.**

Serial dilutions of antibodies were incubated for 6 hours at 37°C with engineered Jurkat effector cells (ADCC Bioassay Effector Cells) and ADCC Bioassay target Cells (IL-13Rα2-positive cells). Luciferase activity was quantified using ONE-Glo™ Luciferase Assay Buffer Reagent. ADCC of LM-106 in human IL-13Rα2-expressing cells **(A)**, endogenous IL-13Rα2 expressing U251 Glioma cancer cells **(B)** and A375 Melanoma cancer cells **(C)** in a dose-dependent manner with EC_{50} of 0.16nM, 0.06nM and 0.03nM, respectively.

LM-306 Has a Target-Dependent Cytotoxicity to Human IL-13Rα2 Expressing Cells

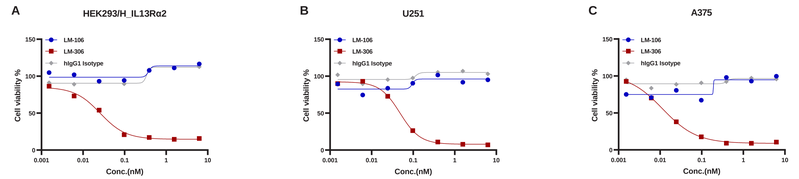


Figure 6: **Target-Dependent Cytotoxic of LM-306.**

IL-13Rα2-positive cells were treated with antibodies at respective concentrations 5 days, and cell viability was determined by CellTiter-Glo® viability assay kit. LM-306 exhibited strong dose dependent cytotoxicity in Human IL-13Rα2 Expressing Cells **(A)** and Endogenous IL-13Rα2 expressing Cancer Cells **(B-C)** with EC_{50} of 0.03nM, 0.05nM and 0.01nM, respectively.

Significant Bystander Effect of LM-306 Toward IL-13Rα2-negative cells

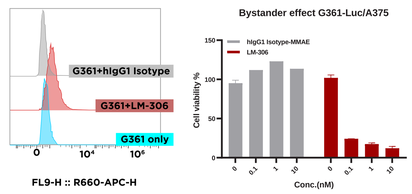


Figure 7: **Bystander Effect of LM-306.**

IL-13Rα2-negative cells G361 (Left) stably expression firefly luciferase as luminescent report gene, named G361-Luc. Endogenous IL-13Rα2 expressing A375 Melanoma cancer cells and G361-Luc cells mixture (ratio=2:1) were seeded to a 96-well plate for 4000 cell/well, and treated with antibodies at 0/0.1/1/10nM for 6 days. The cell viability was evaluated by Cell-Titer-Glo® viability assay kit. Significantly bystander effect was observed when cell mixture was incubated with LM-306 at 0.1/1/10nM, compared to human IgG1 control antibody **(Right).**

LM-306 Reduces Tumors in A375 CDX Model

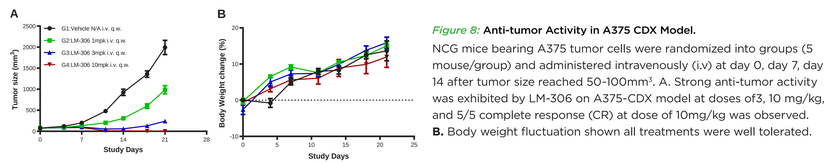


Figure 8: **Anti-tumor Activity in A375 CDX Model.**

NCG mice bearing A375 tumor cells were randomized into groups (5 mouse/group) and administered intravenously (i.v) at day 0, day 7, day 14 after tumor size reached 50-100mm³. A. Strong anti-tumor activity was exhibited by LM-306 on A375-CDX model at doses of3, 10 mg/kg, and 5/5 complete response (CR) at dose of 10mg/kg was observed. **B.** Body weight fluctuation shown all treatments were well tolerated.

Preclinical Nonhuman Primate (NHP) Study

- The toxicity of LM-306 was evaluated in a single intravenous maximum tolerated study (MTD) (18 mg/kg, n = 1/sex/group) and a 4-weeks repeated-dose (Q2W x 3) GLP toxicity study (9 mg/kg, n = 1/sex/group) in cynomolgus monkeys.
- Based on the results, MTD level was considered to be 18 mg/kg for male monkey and 9 mg/kg for female monkey, and 9 mg/kg was considered as HNSTD (highest non-severely toxic dose) level in 4-weeks repeated dose GLP study.

Conclusion

- LM-306 exhibited preclinical efficacy in *in vitro* and *in vivo* studies, along with well safe and tolerable in NHP study.
- LM-306 could significantly inhibit tumor growth, thereby suggesting its role as an anti-tumor agent in cancer therapy.
- Our results show great potential of anti-IL13Rα2-ADC LM-306 as a targeted anti-cancer agent.

Reference

1. Hayato et al. Sci Rep. (2019) 9: 1281.
2. Takahiro et al. *Journal of Urology* (2020) Volume 203 Issue Suppl 4.
3. Bart et al. (2014) Neuro-Oncology 16(10): 1304-1312.
4. Marei et al. Cancer Cell International (2022) 22:255

Disclosures

Disclosures: The authors declare no conflict of interest

Fundings: This study was sponsored by LaNova Medicines, Shanghai, China