LNCB74 is a B7-H4 targeting antibody-drug-conjugate with a β-glucuronide linker-MMAE payload system to enhance the therapeutic index in B7-H4 expressing cancers

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Abstract #1051

Background

LNCB74 is a B7-H4 antibody conjugated to the microtubule disrupting payload monomethyl auristatin E (MMAE) with a drug-to-antibody ratio of 4 (DAR4). The ADC employs a glucuronidase-cleavable, site-specific linkage conjugated to an engineered cysteine in the antibody light chain via LigaChem Biosciences' ConjuAll™ technology to increase stability in circulation, improve selective release of payload in tumor cells, and reduce payload release in non-tumor cells. LNCB74 incorporates an Fc mitigating mutation to minimize binding and uptake of LNCB74 by Fc receptor expressing immune cells. The ConjuAll technology, with its selective cleavage and release within tumor cells, combined with mitigation of off-target uptake via reduced Fc interactions, is proposed to improve the safety profile and therapeutic index of LNCB74 compared to other B7-H4 targeted ADCs.

Mechanism of Action for LNCB74, a B7-H4 Targeting ADC

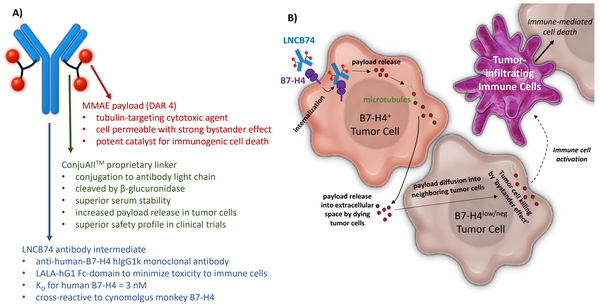


Figure 1. A) Structure of B7-H4 targeting ADC LNCB74. B) Mechanism of action of LNCB74, an ADC which delivers the microtubule-targeting MMAE payload to B7-H4 positive tumor cells. MMAE generates immunogenic cell death and mediates a potent bystander effect to kill neighboring tumor cells following uptake by B7-H4 expressing tumor cells.

B7-H4 is Expressed in Multiple Tumor Indications

B7-H4 is highly expressed in breast, ovarian, and endometrial cancers, as well as in subsets of other cancer types. The limited expression of B7-H4 in healthy cells and tissues makes it an appealing target for antibody-drug-conjugate (ADC) therapeutics.

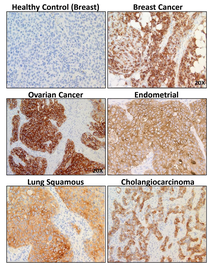


Figure 2. Expression of B7-H4 in cancer. Representative IHC for protein expression in normal breast tissue, and in solid tumor indications with highest prevalence of B7-H4 expression, including breast, ovarian, endometrial, lung and cholangiocarcinoma.

Results

LNCB74 Antibody Intermediate Demonstrates Minimal Fc-Driven Immune Cell Engagement

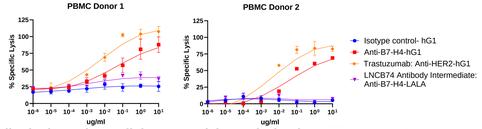


Figure 3. Antibody-dependent cellular cytotoxicity analysis of LNCB74. An ADCC assay was performed on SKBR3 (B7-H4+ HER2+) tumor cells co-cultured with human PBMCs. The LNCB74 antibody intermediate , which contains a LALA mutation to decrease binding of the Fc-domain, was tested against an hG1 bearing anti-B7-H4 antibody and Trastuzumab which has documented ADCC activity.

LNCB74 Antibody Intermediate Specifically Binds and Internalizes into B7-H4 Expressing Tumor Cells

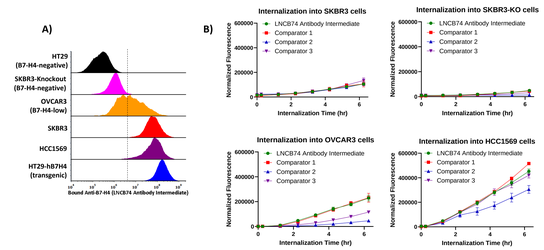
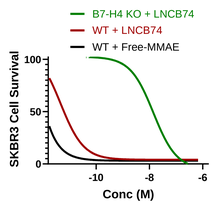


Figure 4. Comparing internalization of LNCB74 and other anti-B7-H4 antibodies. A) Flow cytometric measurement of LNCB74 antibody intermediate binding to B7-H4-negative and positive cell lines. B) Kinetics of internalization of LNCB74 antibody intermediate and comparator B7-H4 antibodies using Fc-glycan-conjugated-pHrodo-labeled antibodies. Relative internalization was measured using an Opera Phenix confocal imaging system.

LNCB74 Mediates Target-Dependent Cytotoxicity *In Vitro*



Cell Line	Tumor Type	B7-H4 Surface Expression	EC50 (nM)	
			B7-H4+	B7-H4-
SKBR3	HER2+ BC	+++	0.005	14
HCC1569	HER2+ BC	+++	2	ND
ZR-75-1	HR+ BC	++	8	ND
OVISE	OC	++/+++	0.1	ND
OVCAR3	OC	+	3	ND
A549-hB7-H4	LC (B7-H4 transgenic)	++++	0.06	713
HT29-hB7-H4	CC (B7-H4 transgenic)	++++	0.2	122

Figure 5. Cytotoxicity assays of LNCB74 in tumor cells from various indications. Representative data showing dose-dependent killing of B7-H4+ SKBR3 breast cancer cells by LNCB74 *in vitro.* Low toxicity to a B7-H4- knockout derivative, and sensitivity of SKBR3 cells to free MMAE toxin are shown for reference.

Table 1. EC50 values for in vitro cytotoxicity by LNCB74 on cancer cell lines. Where possible, EC50 values are shown for isogenic B7-H4-negative cell lines (knockout-derivative or parental for transgenic cell lines).

LNCB74 Elicits Strong Tumor Killing in CDX Models

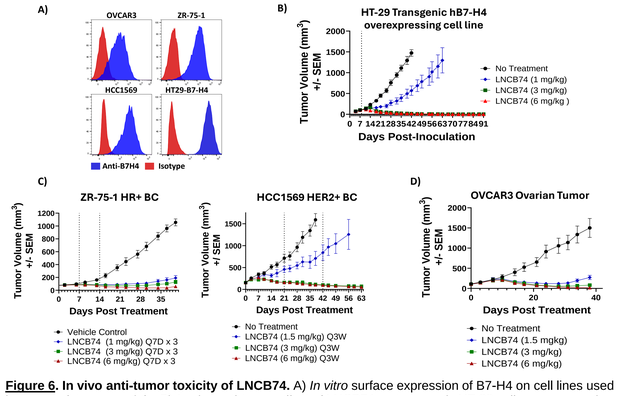


Figure 6. In vivo anti-tumor toxicity of LNCB74. A) *In vitro* surface expression of B7-H4 on cell lines used in xenograft tumor models. B) *In vivo* anti-tumor effect of LNCB74 on transgenic HT-29 cells overexpressing human B7-H4. C) Anti-tumor effect of LNCB74 in the ZR-75-1 and HCC1569 orthotopic breast cancer tumor models. D) Anti-tumor effect of LNCB74 in the OVCAR3 ovarian model. Dashed line indicates day of dosing.

Bystander Effect Demonstrated in CDX Models

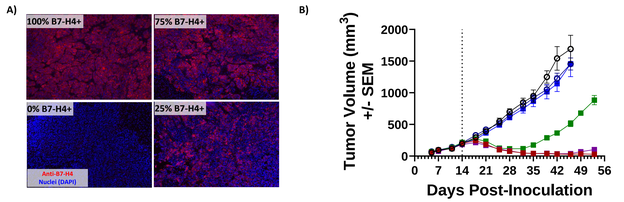


Figure 7. Analysis of bystander activity in lower P-value tumors. A) Immunofluorescent staining of cryosections from xenograft mouse tumors (collected on treatment 'Day 0'). Tumor models were generated with 100%, ~75%, ~25%, or 0% B7-H4 positive cells by mixing transgenic HT29-hB7-H4 cells with B7-H4-negative HT29 'wildtype' cells. B) Dose response for LNCB74 anti-tumor activity using an HT29-hB7-H4 transgenic tumor model demonstrating an anti-tumor/bystander effect of a single dose of 3 mg/kg LNCB74 in a 'mixed' models composed of 100%, 75%, or 25% HT29 B7-H4+ cells.

Potent Anti-Tumor Activity in Breast Cancer PDX Models

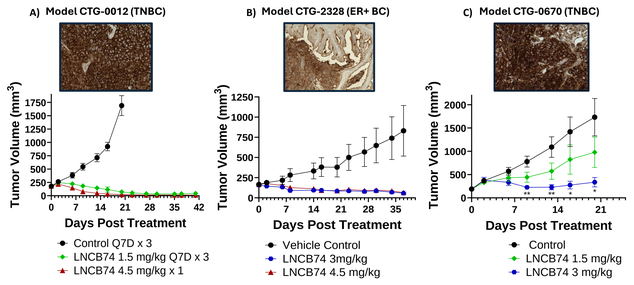


Figure 8. Activity of LNCB74 in three B7-H4-positive PDX models. A) Durable regression was observed in TNBC model CTG-0012 following fractionated dosing of 1.5 mg/kg weekly x 3 as well as with a single bolus of 4.5 mg/kg. B) In HR+BC model CTG-2328, a single dose of 3 mg/kg or 4.5 mg/kg dose was effective. C) In TNBC model CTG-0670, a single dose of 3 mg/kg resulted in control of tumor growth. IHC image above each graph is the IHC demonstrating the presence of B7-H4 expression within the model.

LNCB74 is More Effective than Comparator B7-H4-MMAE

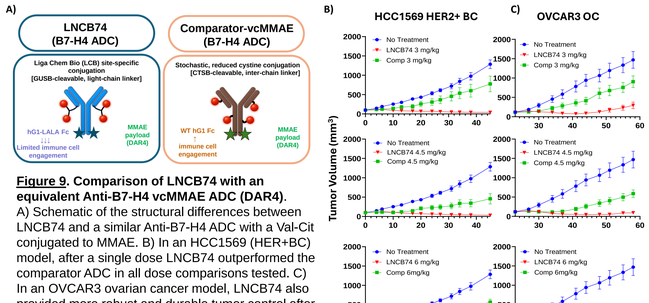
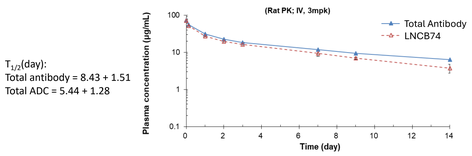


Figure 9. Comparison of LNCB74 with an equivalent Anti-B7-H4 vcMMAE ADC (DAR4). A) Schematic of the structural differences between LNCB74 and a similar Anti-B7-H4 ADC with a Val-Cit conjugated to MMAE. B) In an HCC1569 (HER+BC) model, after a single dose LNCB74 outperformed the comparator ADC in all dose comparisons tested. C) In an OVCAR3 ovarian cancer model, LNCB74 also provided more robust and durable tumor control after a single dose when compared to the comparator anti-B7-H4 ADC with Val-Cit linker, despite both having a DAR4 MMAE payload.

LNCB74 Has a Favorable PK Profile and Serum Stability in Rodent Models



$T_{1/2}$(day):
Total antibody = 8.43 + 1.51
Total ADC = 5.44 + 1.28

Figure 10. PK analysis (LC/MS/MS) of LNCB74 ADC and Total Antibody in rats following single dose administration at a dose of 3 mg/kg, *i.v.*

			DAR change			Toxin release			Toxin release		
LNCB74	Species	Incubation Conc. (µM)	Incubation time (Day)	% Remaining of LNCB74			Concentration of MMAE (nM)			% Release of MMAE		
				Mean	±	SD	Mean	±	SD	Mean	±	SD
LCB14-5302 B7H4 (MMAE/DAR4)	Rat	1	0	100	±	0.00	0.00	±	0.00	0.00	±	0.00
			1	101	±	3.38	15.7	±	0.69	0.39	±	0.02
			3	102	±	3.19	42.3	±	2.27	1.06	±	0.06
			7	93.1	±	7.30	70.8	±	2.46	1.77	±	0.06

Table 2. Plasma Stability of LNCB74 ADC and MMAE toxin release in rats.

LNCB74 is Well Tolerated in Cynomolgus Monkeys

LNCB74 has been shown to be safe and tolerable in toxicity studies. The ADC was dosed up to 10 mg/kg in a pilot toxicity study in cynomolgus monkeys. Observations were limited and minimal, with all animals surviving to the end of the study. This safety profile is expected to translate into tolerability in the clinic.

Conclusions

- B7-H4 protein is highly expressed in multiple tumor indications. B7-H4 expression is low and limited in normal healthy human tissues, providing a potential broad therapeutic index for a B7-H4 targeting ADC
- LNCB74 antibody intermediate is a high-affinity monoclonal hIgG1 (LALA-mutant-Fc) with specificity for human B7-H4. LNCB74 shows specific binding to B7-H4 expressing tumor cells and is rapidly internalized in a target-dependent manner by cancer cells but shows limited interaction with immune cells via its Fc Domain
- LNCB74 is a DAR4 ADC carrying an MMAE tubulin-targeting payload. MMAE was conjugated to the light-chain of the antibody intermediate using LCB's proprietary ConjuAll linker technology
- LNCB74 mediates potent cytotoxicity, with sub-nanomolar to low nanomolar EC50 values on multiple B7-H4-positive cancer cell lines
- LNCB74 shows strong anti-tumor activity in multiple CDX and PDX tumor models. A single dose of 3 mg/kg resulted in durable tumor regression in multiple tumor models, suggesting activity comparable or superior to published B7-H4 targeting ADCs
- LNCB74 demonstrates favorable PK and stability in rodents, with very low release of free MMAE payload in blood, consistent with the safe profile observed preclinically
- LNCB74 was well tolerated in cynomolgus monkeys following 2 doses of up to 10 mg/kg, suggesting an excellent safety profile
- LNCB74 is a promising candidate therapeutic for treating multiple solid tumor indications, including breast, ovarian, and endometrial cancers