LNCB74 is a potent and safe next-generation antibody-drug-conjugate utilizing a cancer selective linker for the treatment of B7-H4 expressing cancers

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Abstract #1898

Background

The B7 family protein B7-H4 is highly expressed on a range of solid tumors including breast, ovarian, and endometrial cancers, where it may play a role in immune evasion. In non-cancerous tissues, B7-H4 expression is limited, suggesting it may be a potential target for an antibody drug conjugate (ADC). LNCB74 is a human IgG1 antibody conjugated to the potent microtubule disrupting payload monomethyl auristatin E (MMAE) with a drug-to-antibody ratio of 4 (DAR4). The ADC employs a highly selective B7-H4 antibody based upon NextCure's expertise in B7-H4 tumor biology coupled with a glucuronidase-cleavable, site-specific linkage to an engineered cysteine in the antibody light chain via LigaChem Biosciences' ConjuAll™ technology. This improves the safety profile and therapeutic index of the agent by a) increasing stability in circulation, b) selectively releasing payload in tumor cells, and c) reducing payload release in off-target cells. LNCB74 was further engineered with a "LALA"-mutant Fc region to minimize uptake and toxicity to immune cells. In a nonhuman primate (NHP) toxicity study, LNCB74 was well tolerated for repeat dosing and provides evidence for a superior safety profile without noticeable side effects. LNCB74 is rapidly internalized by B7-H4-expressing tumor cells, and demonstrates potent, target-specific cytotoxicity on multiple cancer cell lines. In vivo studies demonstrate a strong bystander effect following a single dose administration of LNCB74. A rapid and durable anti-tumor response has been demonstrated in multiple cell-line derived (CDX) and patient-derived xenograft (PDX) tumor models. In summary, LNCB74 is a promising ADC enabling specific targeting of B7-H4 positive cancers across a spectrum of indications.

B7-H4 Protein is Expressed in Multiple Tumor Indications

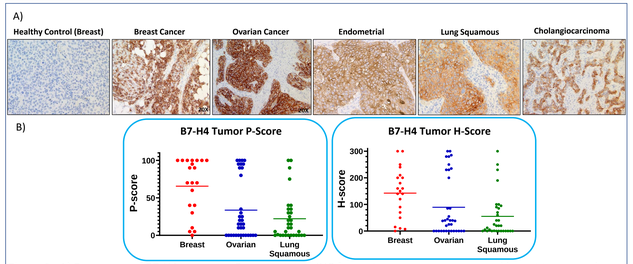

Figure 1. A) Expression of B7-H4 in cancer. Representative IHC for protein expression in normal breast tissue, and in solid tumor indications with highest prevalence of B7-H4 expression, including breast, ovarian, endometrial, lung and cholangiocarcinoma. B) Range of B7-H4 expression in specific indications as determined by IHC analysis of patient-derived tumor microarrays.

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

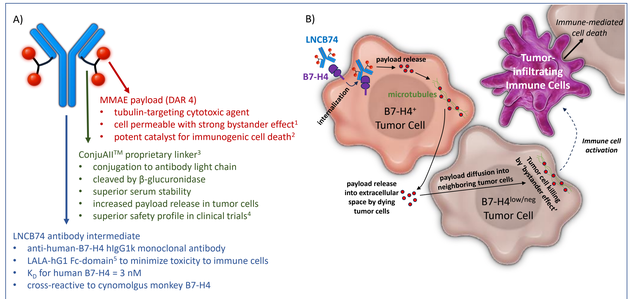

Figure 2. A) Structure of B7-H4 targeting ADC LNCB74. B) Mechanism of action of LNCB74, an ADC which delivers the microtubule-targeting MMAE payload to B7-H4 positive tumor cells. MMAE generates immunogenic cell death and mediates a potent bystander effect to kill neighboring tumor cells following uptake by B7-H4 expressing tumor cells.

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

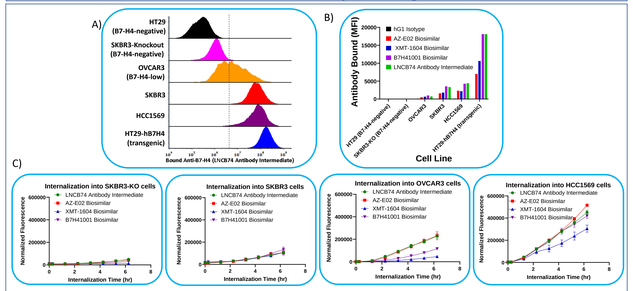

Figure 3. A) Flow cytometric measurement of LNCB74 antibody intermediate binding to B7-H4-negative and positive cell lines. B) Comparison of binding of LNCB74 antibody intermediate vs. 3 published anti-B7H4 monoclonal antibodies[6,7,8] to a panel of B7-H4 negative and positive cell lines using flow cytometry. Site-click technology was used to generate site-specific (Fc-glycan) fluorophore-conjugates for side-by-side comparison. C) 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 Potent, Target-Dependent Cytotoxicity *In Vitro*

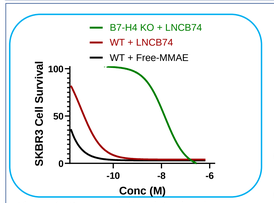

Figure 4. Representative data showing dose-dependent killing of B7-H4+ SKBR3 breast cancer cells by LNCB74 *in vitro*. Low toxicity to a B7-H4- knockout derivative, and sensitivity of SKBR3 cells to free MMAE toxin are shown for reference.

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).

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

LNCB74 Elicits an Anti-Tumor Effect in B7-H4 Low and High CDX Xenograft Tumor Models

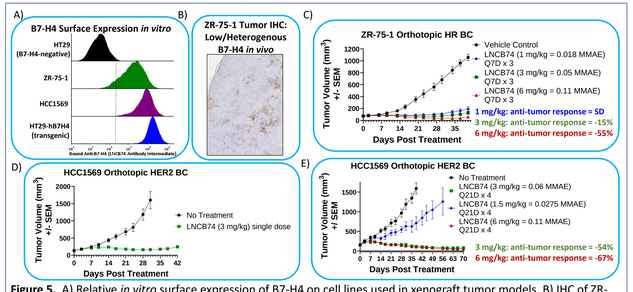

Figure 5. A) Relative *in vitro* surface expression of B7-H4 on cell lines used in xenograft tumor models. B) IHC of ZR-75-1 orthotopic tumors reveals low, heterogenous expression in tumor cells (p-score <<50%). C) Anti-tumor effect of LNCB74 in the ZR-75-1 orthotopic breast cancer tumor model. D) Anti-tumor effect of LNCB74 in the HCC1569 orthotopic breast cancer tumor model following a single dose or E) repeated dosing every 3 weeks. (SD = stable disease)

LNCB74 Demonstrates a Potent Bystander Effect in CDX Xenograft Tumor Models

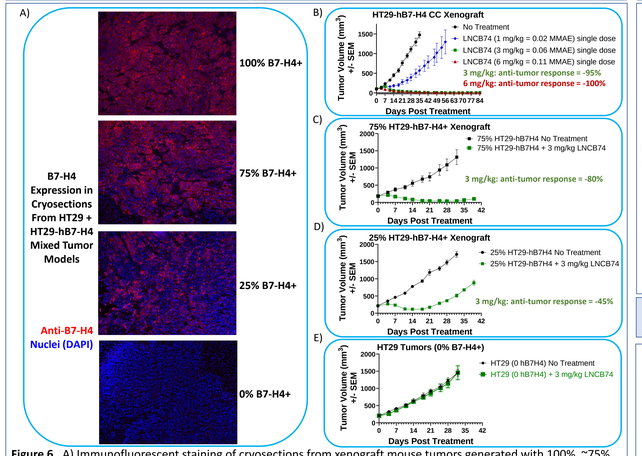

Figure 6. A) Immunofluorescent staining of cryosections from xenograft mouse tumors generated with 100%, ~75%, ~25%, or 0% B7-H4 positive cells by mixing transgenic HT29-hB7-H4 cells with B7-H4-negative HT29 'wildtype' cells. B) Dose response for LNCB74 anti-tumor activity using an HT29-hB7-H4 transgenic tumor model. C) Anti-tumor / bystander effect of a single dose of 3 mg/kg LNCB74 in a model composed of 75% B7-H4+ cells. D) Anti-tumor / bystander effect of a single dose of 3 mg/kg LNCB74 in a model composed of 25% B7-H4+ cells. E) LNCB74 does not affect tumor progression in HT29 tumors which do not express B7-H4 target protein.

LNCB74 Shows Potent Anti-Tumor Activity in 3 Breast Cancer PDX Models

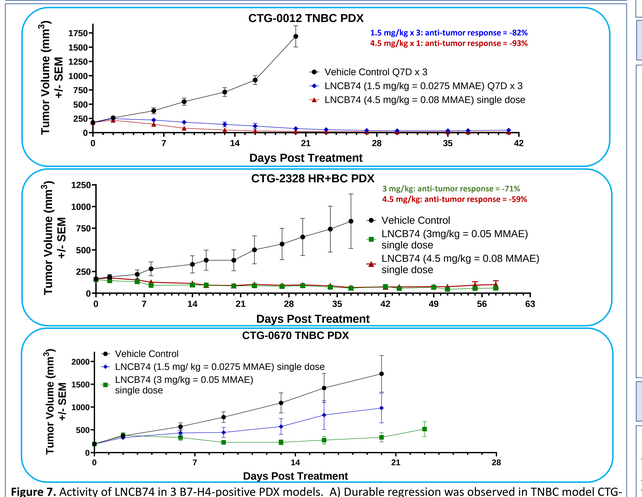

Figure 7. Activity of LNCB74 in 3 B7-H4-positive PDX models. A) Durable regression was observed in TNBC model CTG-0012 following fractionated dosing of 1.5 mg/kg weekly x 3. A single bolus of 4.5 mg/kg showed even greater efficacy. B) In HR+BC model CTG-2328, a single dose of 3 mg/kg was as effective as a 4.5 mg/kg dose. C) In TNBC model CTG-0670, a single dose of 3 mg/kg resulted in tumor growth control.

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

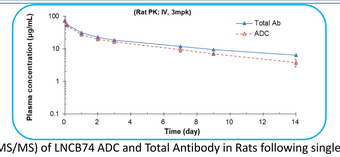

Figure 8. PK analysis (LC/MS/MS) of LNCB74 ADC and Total Antibody in Rats following single dose administration at a dose of 3 mg/kg, *i.v.*

Table 2. Serum Stability of LNCB74 ADC and MMAE toxin release in rat serum.

				DAR change				Toxin release				Toxin release			
				% Remaining of ADC				Concentration of MMAE (nM)				% Release of MMAE			
ADC	Species	Incubation Conc. (μM)	Incubation time (Day)	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	

LNCB74 is Well Tolerated in Cynomolgus Monkeys

Table 3. Design and results from a pilot study to access toxicity of LNCB74 in cynomolgus monkeys. No mortalities or major toxicities were observed in either study. Typical MMAE-associated events (neutropenia, RBC decrease) were observed with recovery by endpoint.

PILOT TOX STUDY	
Dose Levels	5 and 10 mg/kg
Dose Administration	Two Q21D doses administered i.v., followed by a 21-day observation period
Macroscopic Observations	• No observations at 5 mg/kg • At 10 mg/kg: – Skin discoloration – Lung adhesion to thoracic wall and diaphragm in one animal (cannot be attributed independently to test article) – Minimal Increase in AST (fully reversable) – Mild dose-dependent decrease in RBC, hemoglobin, hematocrit – Dose-dependent changes to WBC, neutrophils observed (fully reversable)
Overall Observations	• All animals survived at day 43 • Safe and tolerable

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
- LNCB74 is a DAR4 ADC carrying a tubulin targeting MMAE payload. MMAE was conjugated to the light-chain of the antibody intermediate using LCB's proprietary linker technology
- LNCB74 mediates potent cytotoxicity, with sub-nanomolar to low nanomolar EC50 values on multiple B7-H4-positive cancer cell lines
- 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 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 was well tolerated in cynomolgus monkeys following 2 doses of up to 10 mg/kg, suggesting a superior safety profile
- LNCB74 is a promising candidate therapeutic for treating multiple solid tumor indications, including breast, ovarian, endometrial, lung, and biliary cancers

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