LCB84, a TROP2–targeted ADC, for treatment of solid tumors that express TROP2 using the Hu2G10 tumor-selective anti–TROP2 monoclonal antibody, a proprietary site-directed conjugation technology and plasma-stable tumor-selective linker chemistry

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Abstract

LCB84 is a human TROP2-targeting antibody drug conjugate (ADC) composed of monomethyl auristatin E (MMAE) as payload and the Hu2G10 (by Mediterranea Theranostic) humanized IgG1 antibody that selectively targets the ADAM10-activated TROP2 protein selectively expressed in transformed cancer cells. LCB84 was prepared using *ConjuAll*™, a proprietary site-directed conjugation technology of LegoChem Biosciences, which incorporates a conjugation 'handle' joined by enzymatic prenylation to a specifically engineered recognition sequence (CaaX) on antibody light chains. This conjugation handle facilitates simple versatile chemical conjugation to the linker-payload. A proprietary plasma-stable cleavable linker that is recognized and cleaved by a cancer-associated lysosomal enzyme, β-glucuronidase, was used to enable efficient and traceless payload release in a cancer-specific manner. LCB84 has been evaluated for anti-tumor activity and showed superior anticancer efficacy in triple-negative breast cancer (TNBC), pancreatic ductal adenocarcinoma (PDAC), gastric cancer and non-small cell lung cancer (NSCLC) cell line-derived xenograft (CDX) models compared to the ADC competitors Trodelvy and DS-1062BS. The LCB84 treatments were well tolerated, with no changes in body weight compared to control animals, for all dosing groups. LCB84 has robust cross-reactivity against primate TROP2, which allows rigorous toxicity studies in monkeys. Remarkably, preliminary toxicity studies using cynomolgus monkeys showed that LCB84 is well tolerated, with calculated therapeutic index (TI, MTD / MED) of ~30 for single dosing and ~40 for repeat dosing. In conclusion, LCB84 is highly effective against TROP2-positive CDX models in mice at doses that are well tolerated in mice and in primate models. Use of this proprietary plasma-stable cancer-selective linker technology and the Hu2G10 anti–TROP2 monoclonal antibody that targets cancer-activated TROP2 has led to a greatly improved next generation ADC for the treatment of various TROP2-positive solid cancers including TNBC, PDAC, NSCLC and gastric cancer.

Introduction

LCB's ADC Platform – *ConjuAll*™

Site-specific ADCs with highly stable, tumor-selective linker
***ConjuAll*™: site-specific, homogeneous, and non-reversible conjugation**

► Primary Objective

- ✓ Site-specific & Homogeneous (exact DAR)
- ✓ Plasma-stable linker (not unstable thiol-maleimide conjugation)
- ✓ Efficient release of toxin inside of the target cancer cell

► Strategy

- ✓ Engineered antibody with extra sequence at C-term (Heavy chain or Light chain)
- ✓ Specific functionalization via prenylation
- ✓ Linker-payload conjugation using the modified prenyl group

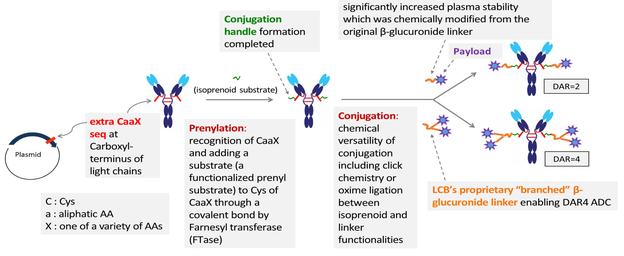
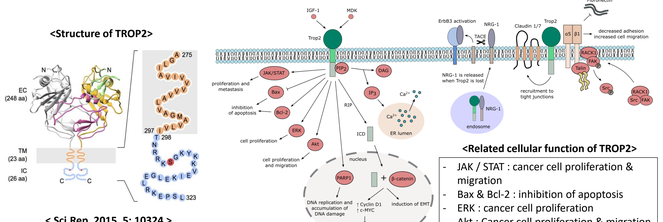


Figure 1. Schematic figure of *ConjuAll*™ technology

Introduction of TROP2

Alias : TACSTD2 (tumor associated calcium signal transducer 2), EGP-1 (epithelial glycoprotein-1)

<Structure of TROP2>



< Sci.Rep. 2015, 5: 10324 >

<Related cellular function of TROP2>

- JAK / STAT : cancer cell proliferation & migration
- Bax & Bcl-2 : inhibition of apoptosis
- ERK : cancer cell proliferation
- Akt : Cancer cell proliferation & migration

< Cancers 2020, 12(11): 3328 >

Figure 2. Structure and cellular function of TROP2

Anti-TROP2 ADC competitors

- ✓ Trodelvy is a product for clinical use and was purchased for research use from Advanced Care Associates LLC (USA) through Eversell Inc. (South Korea).
- ✓ DS-1062BS was manufactured as a biosimilar by a CRO located in the United States based on patent No. US10,227,417B2 and US11,173,213B2.

Results

Cancer cell binding & internalization of hu2G10 CaaX body

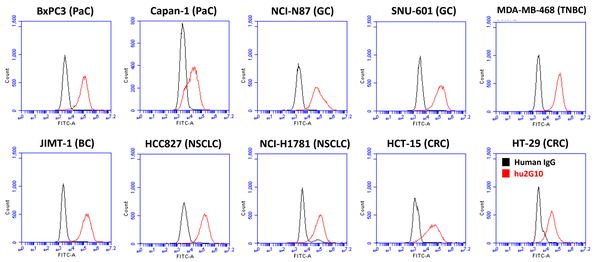


Figure 1. FACS analysis in various cancer cell lines including pancreatic cancer (BxPC3, Capan-1), gastric cancer (NCI-N87, SNU-601), triple-negative breast cancer (MDA-MB-468), breast cancer (JIMT-1), non-small cell lung cancer (HCC827, NCI-H1781) and colorectal cancer (HCT-15, HT-29). Humanized anti-cleaved TROP2 mAb (hu2G10) showed strong binding with various cancer cell lines.

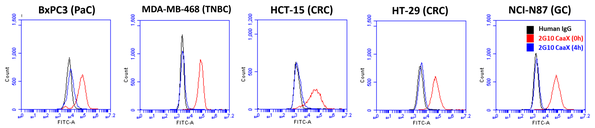


Figure 2. Internalization of hu2G10 mAb in various cancer cell lines including pancreatic cancer (BxPC3), triple-negative breast cancer (MDA-MB-468), colorectal cancer (HCT-15, HT-29, DLD-1) and gastric cancer (NCI-N87). Most of humanized anti-cleaved TROP2 mAb (hu2G10) was confirmed to be internalized within 4 hours.

Table 1. Summary of internalization of hu2G10 mAb

Cells	BxPC3	MDA-MB-468	HCT-15	HT-29	NCI-N87
Internalization of hu2G10 mAb (by FACS)					
0h	71,622	80,996	36,070	55,687	73,205
4h	2,690	100	251	906	100
Internalized (%)	96.2%	99.9%	99.3%	98.4%	99.9%

In vitro cytotoxicity of LCB84 in various cancer cells

Table 2. *In vitro* cytotoxicity of LCB84 after a 144-hour exposure on various solid cancer cells, as measured by the Sulforhodamine B (SRB) colorimetric cell viability assay. LCB84 showed potent cytotoxicity in various solid cancer cells. Pancreatic cancer (BxPC-3, Capan-1, Patu8988s), gastric cancer (NCI-N87, SNU-601), triple-negative breast cancer (MDA-MB-468), breast cancer (JIMT-1), non-small cell lung cancer (HCC827, NCI-H1781, Calu-3), ovarian cancer (OVCAR-3), colorectal cancer (HT-29, COLO205).

	CC_{50} (nM)						
Test sample	**BxPC-3**	**Capan-1**	**Patu8988s**	**NCI-N87**	**SNU-601**	**MDA-MB-468**	**JIMT-1**
MMAE	0.410	0.520	0.334	0.605	0.2682	0.660	0.112
LCB84	1.493	18.060	1.412	0.584	7.267	3.293	0.111
Test sample	**HCC827**	**NCI-H1781**	**Calu-3**	**OVCAR-3**	**HT-29**	**COLO205**	
MMAE	0.622	0.208	0.400	0.288	0.541	0.628	
LCB84	11.290	3.22	3.538	7.33	2.419	11.950	

In vitro cytotoxicity of LCB84 in various normal cells

Table 3. *In vitro* cytotoxicity of LCB84 after a 144-hour exposure on various normal cells, as measured by the CellTiter-Glo cell viability assay or the Sulforhodamine B (SRB) colorimetric cell viability assay. LCB84 was quite tolerable in various human normal cells. Fa2N-4, normal liver cell; HK-2, normal human kidney cell; Hs738.st/Int, normal gastrointestinal cell; Detroit 551, normal skin cell.

	CC_{50} (nM)				
Test sample	**Naïve hPBMC**	**Fa2N-4**	**HK-2**	**Hs738.st/Int**	**Detroit 551**
MMAE	110.0	0.41	21.9	> 500 (54.1%)	7.83
LCB84	> 200 (81.4%)	166.3	> 500 (69.4%)	> 500 (75.8%)	> 500 (63.3%)

* The parentheses indicate cell viability at the corresponding concentration

In vivo efficacy of LCB84 in various solid cancer xenograft models

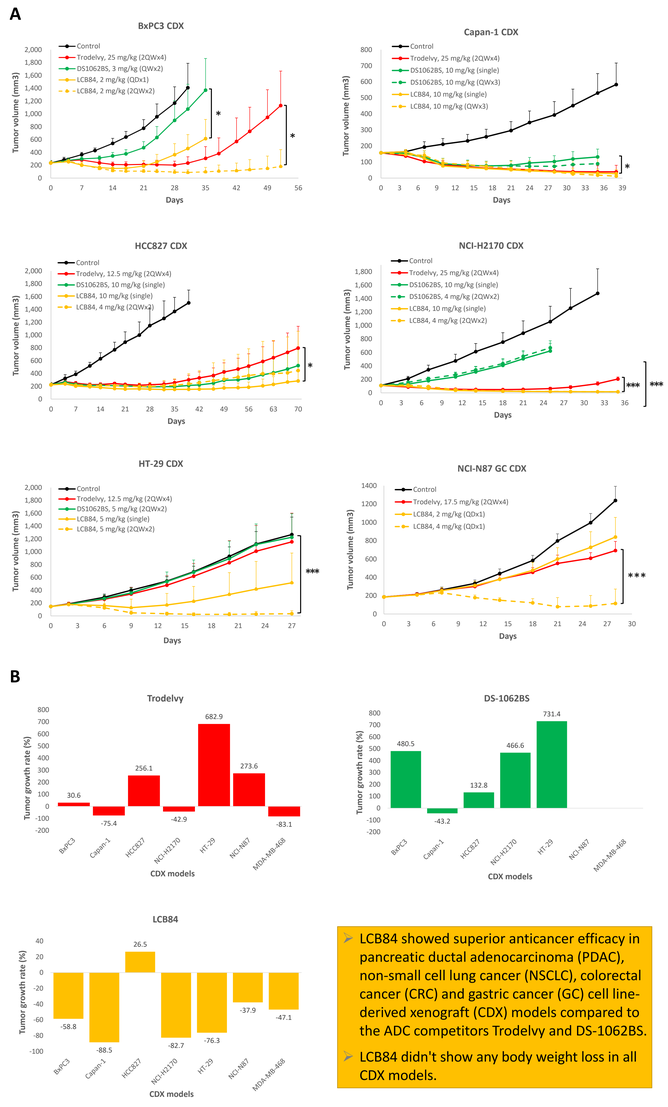


Figure 3. Tumor volume change (A) and tumor growth rate comparison (B) in various *in vivo* xenograft models. Trodelvy, DS-1062BS and LCB84 were administrated intravenously to balb/c nude mice containing various SC xenografts. Trodelvy, DS-1062BS and LCB84 were injected at the doses described in the graph (in LCB : BxPC3, HCC827 CDX models; in CRO : Capan-1, NCK-H2170, HT-29, NCI-N87 CDX models). Each value of tumor volume represents the mean and SD (N=5 in LCB and N=8 in CRO), and statistically significant difference between LCB84 and competitors analyzed by unpaired *t* test (*, P < 0.05; ***, P < 0.001).

Therapeutic index comparison of TROP2-ADCs

Table 4. Therapeutic index of 3 anti-TROP2 ADC

TROP-2 ADC	Minimum Efficacious Dose (MED, mouse xenografts)	Maximum Tolerated Dose (HNSTD NHPs)	Therapeutic Index
Trodelvy (approved) (Trop2-SN38)	25 mg/kg (2QWx4) 75 mg/m^2	60 mg/kg (reported) 720 mg/m^2	2.4 (mg/kg basis) 9.6 (mg/m^2 basis)
DS-1062 (Trop2-Dxd)	10 mg/kg (single) 30 mg/m^2	10 mg/kg (reported) 120 mg/m^2	1 (mg/kg basis); 4 (mg/m^2 basis)
LCB 84 (Trop2-MMAE)	2 mg/kg (single) 6 mg/m^2	10 mg/kg (tested) 120 mg/m^2	5 (mg/kg basis) 20 (mg/m^2 basis)

* LCB judges as MED if the tumor size on the 21st day after the last administration of ADC is less than the tumor size on day 0.

- *In vivo* efficacy study, commercially available Trodelvy was used, and it was confirmed that it showed efficacy similar to that of a previously published patent or research papers.
- However, DS-1062 is a material that has not been approved yet, so based on the published patent, it was manufactured by CRO (USA) in the form of a biosimilar (DS-1062BS) and used for *in vivo* efficacy study. DS-1062BS showed good efficacy similar to the result from the previously published paper in the HCC827 CDX model, but not in the NCI-H2170 CDX model. This is probably because it is not the exactly same material as DS1602 or because of the difference in the characteristics of the NCI-H2170 cell line used by CRO (China).
- The MED of Trodelvy and DS-1062 described in the table above is the dose confirmed in the animal efficacy comparison study conducted by LCB or CRO (China), and the HNSTD of NHP is extracted from the EMA approved document or published papers.
- LCB84 has the best-in-class TI compared to all advanced anti-Trop-2 ADC molecules without target (Trop2) based toxicities (e.g., skin toxicity, neutropenia, etc.) in monkeys

- LCB84 showed superior anticancer efficacy in pancreatic ductal adenocarcinoma (PDAC), non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and gastric cancer (GC) cell line-derived xenograft (CDX) models compared to the ADC competitors Trodelvy and DS-1062BS.
- LCB84 didn't show any body weight loss in all CDX models.

Summary

- LCB84 is an ADC prepared by *ConjuAll*™, a proprietary site-specific conjugation technology of LegoChem Biosciences and a novel tumor-selective TROP2-targeting antibody from Mediterranea Theranostic. LCB's BG (β-glucuronidase) linker was also utilized to come up with LCB84.
- *In vitro*, LCB84 shows excellent potency in TROP2-expressing solid cancer cell lines including pancreatic ductal adenocarcinoma (PDAC), non-small cell lung cancer (NSCLC), gastric cancer (GC), triple-negative breast cancer (TNBC), ovarian cancer (OvC) and colorectal cancer (CRC).
- *In vivo*, LCB84 shows superior anticancer efficacy in pancreatic ductal adenocarcinoma (PDAC), non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and gastric cancer (GC) cell line-derived xenograft (CDX) models compared to the ADC competitors Trodelvy and DS-1062BS.
- LCB84 also showed superior anticancer efficacy in TNBC CDX model, such as MED was confirmed as a single dose of 2 mg/kg and repeated doses of 1 mg/kg. Efficacy comparison study with Trodelvy and DS-1062BS are currently in progress.
- In cynomolgus monkey toxicology study, repeated doses of LCB84 at 10 mg/kg is well tolerated. Skin toxicity seen in competitors and hematological toxicity such as neutropenia and thrombocytopenia, which are known as general toxicity of ADC, were not observed in tested doses.
- Using LegoChem's conjugation technology and tumor-selective anti-TROP2 antibody, LCB84 is a promising ADC for the treatment of various solid cancer including TNBC, NSCLC, PDAC, GC, OvC and CRC.

References

1. Kim *et al.*, Antibody-active agent conjugates and methods of use. *US 9 669 107 B2*
2. Guerra, E., Alberti, S., Humanized anti-TROP2 monoclonal antibodies and uses thereof. *EP 3 227 340 B1*
3. Guerra, E., Alberti, S., Humanized anti-TROP2 monoclonal antibodies and uses thereof. *US 10 501 555 B2*
4. Marco Trerotola *et al.*, Trop-2 cleavage by ADAM10 is an activator switch for cancer growth and metastasis. NEOPLASIA, 2021. 23(4): 415-428.
5. Miha et al., The cytosolic tail of the tumor marker protein Trop2 – a structural switch triggered by phosphorylation. Sci Rep, 2015. 5:10324
6. Sara et al., Trop2: Jack of all trades, master of none. Cancers, 2020. 12(11): 3328.
7. Yamaguchi *et al.*, Method for producing antibody-drug conjugate. *US 2020/0385422 A1*
8. Noguchi *et al.*, Method for selectively manufacturing antibody-drug conjugate. *US 11 173 213 B2*
9. Agatsuma *et al.*, anti-TROP2 antibody-drug conjugate. *US 10 227 417 B2*
10. Daisuke *et al.*, Datopotamab deruxtecan, a novel TROP2-directed antibody-drug conjugate, demonstrates potent antitumor activity by efficient drug delivery to tumor cells. Mol Cancer Ther, 2021. 20(12): 2329-2340
11. Assessment report of Trodelvy. EMA, 2021. 623887