Preclinical Characterization of Novel LIV1 Antibody Drug Conjugates

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Poster#4441

Background

- LIV1 is a member of the zinc transporter family. With limited normal tissue expression, LIV1 was found to be overexpressed with high prevalence in breast (93%), prostate (72%) and lung (10%) cancers, and considered as an attractive cell surface target for developing ADC therapeutics.
- We have generated 48D6, a proprietary novel humanized anti-LIV1 mAb with high affinity, specificity, internalization ability, unique epitope and improved pharmacokinetics (PK) profile in mice.
- Then we developed 48D6 based ADCs using glycotransferase mediated site-specific conjugation with Topo I inhibitor (ADC-2) or MMAE (ADC-3), and characterized their anti-tumor activities in preclinical PDX/CDX models and exploratory toxicity in mice.

Methods

- PK properties were studied with single dose (i.v.) of 3 or 10 mg/kg of both naked antibodies and ADCs in Balb/c mice.
- To evaluate anti-tumor activities, nude mice were subcutaneously implanted with LIV1-expressing PDX tumor blocks. When tumors reached ~200mm3, mice were treated (i.v.) with isotype control-ADC or LIV1 ADCs.
- For exploratory tox study, mice were i.v. injected with LIV1 ADC or 48D6 at 10, 30, 60 mg/kg every week for 3 times, then recovered for 4 weeks.

The conjugation structure of ADC-1, ADC-2 and ADC-3

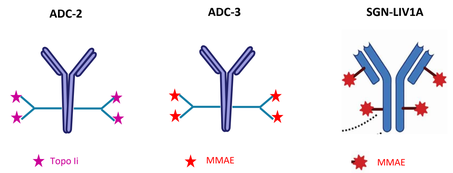
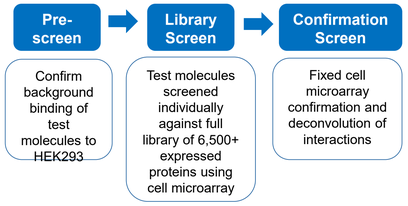


Figure 1. ADC-2 and ADC-3 were generated by using site-specific conjugation technology with 2 different payloads, while SGN-LIV1A analog was using cysteine based random conjugation[1].

48D6 has no non-specificity interaction with other human proteins

A



B

Gene ID	Median SNR	
	48D6 (2 mg/mL)	Rituximab biosimilar (1 mg/mL)
FLRT1	-0.53	-0.15
FCGR2A (Isoform 2)	3.32	6.03
WWP1	-0.14	0.21
FCGR2A (Isoform 1)	3.51	5.35
FCGR3A + FCER1G	4.26	6.47
FCGR3A + CD247	2.69	3.61
CDH8	-0.38	0.04
CD20	-0.32	4.98
EGFR	-0.45	-0.14

Figure 2. Specificity study of 48D6 using Retrogenix cell microarray technology.
A. Process of non-target binding screen. B. Results of the library and confirmation screens. SNR: signal to noise ratio (normalized against secondary only control). Green box: significant interaction (SNR≥1) with differing FCGR family members, which are presumably Fc domain mediated; Red box: positive control interaction.

ADC-2 displays better PK than SGN-LIV1A in Balb/c mice

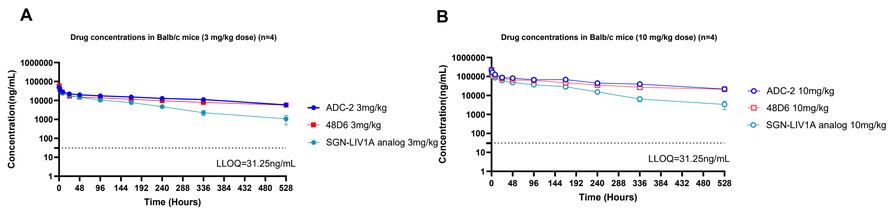
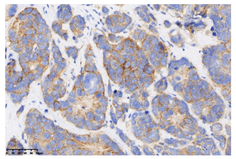


Figure 3. Single dose pharmacokinetic and stability study of ADC-2 and 48D6 in Balb/c mice.
Drug concentration of ADC-2 was measured as total IgG concentration. The half lifer (T1/2) of of ADC-2 is 10.4~11.6 days relative to 3.7~3.9 days for SGN-LIV1A analog, and is quite similar with that of naked Ab 48D6 (T1/2 is 13.8~15.6 days) in Balb/c mice, indicating the good *in vivo* stability of ADC-2 in mice.

ADC-2 exhibited potent anti-tumor activity in breast PDX models *in vivo*

A



Biomarkers and SOC
ER (40%, +), PR (0), HER2 (0), Resistant to Fulvestrant (estrogen antagonist) and Palbociclib (inhibitor of Cdk4 and Cdk6), PD-L1 (-)

B

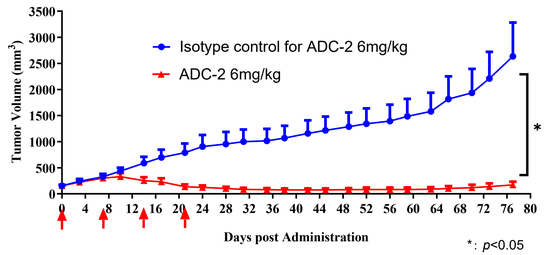
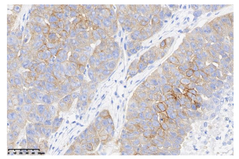


Figure 4. Efficacy of ADC-2 on two breast PDX model at 6 mg/kg.
A. LD1-2009-362153 is an ER+ breast PDX with LIV1 expression between 50% and 75%, detected by IHC. The photo magnification is 40X. B. Nude mice were inoculated with small tumor blocks. LIV1 expression level was detected after inoculation but before grouping by IHC. When tumor size was around 200^mm3. mice were grouped and i.v. injected with ADCs once a week for 4 weeks (red arrows). Average tumor volume (±SEM) were measured twice a week.

ADC-2 exhibited potent anti-tumor activity in NSCLC PDX models *in vivo*

A



Biomarkers and SOC
Lung adenocarcinoma with ALK mutation. EGFR (-), KRAS (-), c-Met (-), TROP2 (-), PD-L1 (+)

B

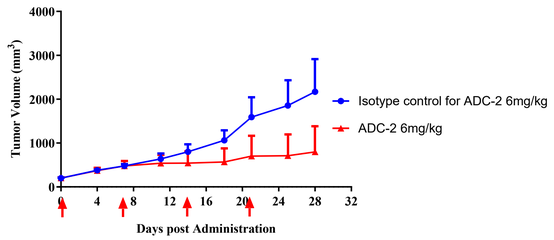
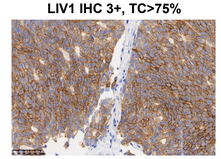


Figure 5. Efficacy of ADC-2 on two lung adenocarcinoma PDX model at 6 mg/kg.
A. LIV1 IHC photo of LD1-0025-215648. The magnification is 40X B. Nude mice were inoculated with small tumor blocks. LIV1 expression level was detected after inoculation but before grouping by IHC. When tumor size was around 200^mm3. mice were grouped and i.v. injected with ADCs once a week for 4 weeks (red arrows represent drug administration). Average tumor volume (±SEM) were measured twice a week.

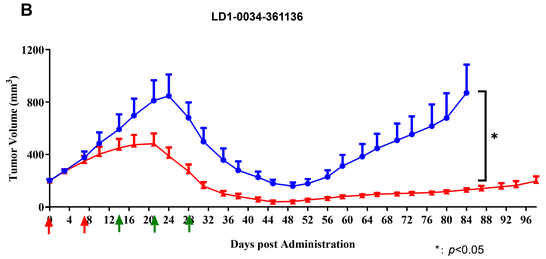
ADC-3 exhibited more potent anti-tumor activity in prostate PDX models *in vivo*

A

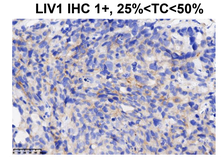


Biomarkers and SOC
Moderately differentiated prostatic ductal adenocarcinoma

B



C



Biomarkers and SOC
Poorly differentiated prostatic ductal adenocarcinoma with focal neuroendocrine features. Used drugs: Abiraterone, Paclitaxel, Goserelin.

D

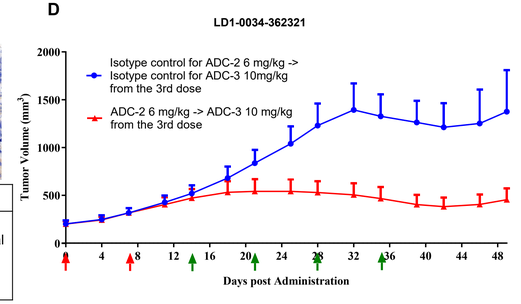


Figure 6. Efficacy of ADC-2 and ADC-3 on two prostate PDX models.
Nude mice were inoculated with small tumor blocks. LIV1 expression level was detected after inoculation but before grouping by IHC (labeled above each graph). When tumor size was around 200^mm3. mice were grouped and i.v. injected with ADC-2 or isotype control for ADC-2 at 6 mg/kg once a week for 2 weeks (red arrows). Then they were replaced by ADC-3 or isotype control for ADC-3 at 10 mg/kg once a week for 3-4 weeks (green arrows). Average tumor volume (±SEM) were measured twice a week. A. LIV1 IHC photo of LD1-0034-361136. The magnification is 40X. B. Tumor growth curve of LD1-0034-361136. C. LIV1 IHC photo of LD1-0034-362321. The magnification is 40X C. Tumor growth curve of LD1-0034-362321.

Results and Conclusions

- Topo I inhibitor payload based ADC-2 displayed strong anti-tumor activities in LIV1 expressing breast and NSCLC PDX models.
- For LIV1 expressing prostate PDX models, two doses of ADC-2 did not inhibit tumor growth significantly. After MMAE-based ADC-3 replaced ADC-2 from the 3rd dose, ADC-3 inhibited the growth of the prostate tumor significantly. In a LIV1 high expressing prostate PDX, the tumor growth was suppressed by ADC-3 for over 70 days after the dosing was stopped on Day 28.
- In exploratory tox study, ADC-2 were well tolerated following repeated administrations in mice at all doses tested. Slight lesions were observed in 60 mg/kg group during the treatment period and fully recovered at the end of the recovery period. Based on these results, the maximum tolerated dose (MTD) of ADC-2 in mice was determined at 60 mg/kg.
- **Conclusions:** LIV1 targeting ADC-2 and ADC-3 exhibited strong anti-tumor activities as monotherapy in PDX models and the anti-tumor activity is further enhanced by checkpoint inhibitor. ADC-2 displayed excellent tolerability profile in mice. These results support further investigation of our LIV1 ADCs in LIV1 positive solid tumors.

References
[1] Sussman D, Smith L M, Anderson M E, et al. SGN–LIV1A: A novel antibody–drug conjugate targeting LIV-1 for the treatment of metastatic breast cancer[J]. Molecular cancer therapeutics, 2014, 13(12): 2991-3000.

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