Novel Humanized LIV-1 Antibody Based ADCs Site-Specifically Conjugated with Topoisomerase I Inhibitor Payloads Displayed Significantly Higher Anti-tumor Activities than MMAE based ADCs in TNBC Tumor Models

Fei Teng[1], Huanhuan Guo[1], Hongjun Li[1], Lizhi Qin[1], Hanjing Mao[1], SonnyYao[1], Xiaoli Zi[1], Lisa Zheng[1], Yi Gu[1], Xueming Qian[1].

[1]Suzhou Transcenta Therapeutics Co., Ltd, Suzhou, China

SAN ANTONIO BREAST CANCER SYMPOSIUM®
DECEMBER 10–13, 2024
SABCS.ORG

Abstract

LIV-1 is a member of the zinc transporter family and an estrogen-regulated gene in metastatic breast cancer. While normal tissue expression is limited, LIV-1 was found to be overexpressed in a high prevalence in breast cancer (93%), as well as in melanoma (82%), prostate (72%), ovarian (48%) and uterine (30%) cancers[1]. LIV-1 is considered as one of the attractive cell surface targets for developing ADC therapeutics. To develop next generation LIV1 targeting ADC, we generated 48D6, a proprietary novel humanized anti-LIV-1 mAb with high affinity, specificity, internalization ability, unique epitope and improved pharmacokinetics profile in mice. *In vitro* studies indicated that breast tumor cells, such as MDA-MB-468 and MCF-7, are more sensitive to Topo I inhibitor than MMAE. Therefore, we generated two Topo I inhibitor-based ADCs (ADC-1 and ADC-2) using glycotransferase mediated site-specific conjugation. Both ADC-1 and ADC-2 have a drug-to-antibody ratio (DAR) of 4 but with two different Topo I inhibitor payloads. A MMAE based ADC (ADC-3) with the same site-specific conjugation and DAR4 was also synthesized as the control. ADC-1 and ADC-2 displayed similar and specific cytotoxic activities against human LIV-1-expressing tumor cells *in vitro*, as compared to SGN-LIV1A analog (DAR4) or ADC-3. In the human LIV-1 transfected MDA-MB-468, a triple-negative breast cancer (TNBC) tumor model, ADC-1 or ADC-2 demonstrated dose-dependent anti-tumor activities and inhibited tumor growth more potently than the SGN-LIV1A analog or ADC-3. At 3 mg/kg the tumor growth inhibition (TGI)% are: ADC-1 92.4%, ADC-2 94.7%, ADC-3 68.5% and SGN-LIV1A analog 57.0% on Day 30; At 3 mg/kg, the overall response rate (ORR, 50% reduction of tumor volume from baseline) of SGN-LIV1A analog or ADC-3 was 0%, while ORRs of ADC-1 and ADC-2 were 40% and 70%, respectively. At 6 mg/kg, on Day 42 ADC-1 and ADC-2 had ORR of 90% and 100% respectively, and CR rate of 90% and 100% respectively. And the body weight of mice didn't change significantly at either 3 or 6 mg/kg for ADC-1 or ADC-2. The enhanced anti-tumor activities of ADC-1 and ADC-2 are likely contributed by the high affinity binding of 48D6 to LIV-1 and high cytotoxicity of Topo I inhibitor in breast tumor cells. These data warrant further investigation of the lead LIV-1 targeting ADCs (ADC-1 and ADC-2) as potential next-generation therapeutic agent in LIV-1 positive breast cancer and other solid tumors.

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

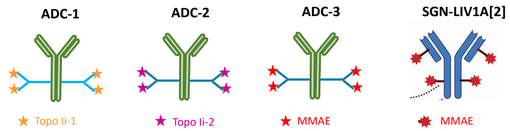

Figure 1. ADC-1, ADC-2 and ADC-3 were generated by using site-specific conjugation technology with 3 different payloads, while SGN-LIV1A analog was using cysteine based random conjugation[1].

High affinity and specificity of ADC-1 and ADC-2

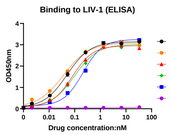

Figure 2. Antibody and ADCs binding to recombinant human LIV-1 (1 μg/ml) measured by ELISA.

Table 1.Non-specific cytotoxicity of ADCs to LIV-1 negative cell lines.

Studies		Breast cancer		CRC	Lung cancer
		MDA-MB-468	T47D	NCI-H716	NCI-H460
LIV-1 expression (IHC)		Negative	Negative	Negative	Negative
Cytotoxicity	ADC-1	Negative	Negative	Negative	Negative
	ADC-2	Negative	Negative	Negative	Negative

48D6 binds to distinct epitope from Ladiratuzumab and has longer half life than Ladiratuzumab in mice

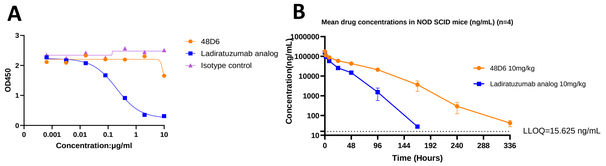

Figure 3. A, 48D6 does not block the binding of Ladiratuzumab analog-biotin to LIV-1. Gradient diluted 48D6 or Ladiratuzumab analog were added to the plate with immobilized LIV-1 before adding 0.1 μg /ml Ladiratuzumab analog-biotin. **B, Single dose pharmacokinetic and stability study of 48D6 and Ladiratuzumab analog in NOD SCID mice.** The t1/2 of 48D6 is about 1.15 days in mice while that of Ladiratuzumab-analog is about 0.56 day.

48D6, ADC-1 and ADC-2 can be internalized into LIV-1 expressing TNBC cell line

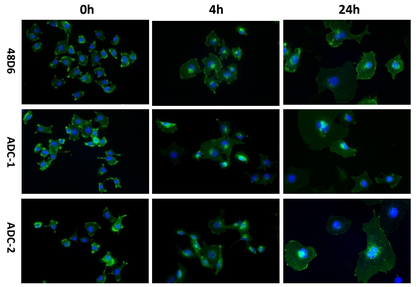

Figure 4. The internalization images of LIV-1 antibody or ADCs by MDA-MB-468-LIV-1 cells. Adherent tumor cells were incubated with 10 μg/ml antibody or ADC for 0, 4, 24 hours at 37℃. Then Ab or ADC were detected with goat anti-human IgG Alexa Fluor488 after cells were fixed and permeabilized. Cells were mounted in ProLong Glod Antifade with DAPI. (Magnification: 400X)

ADC-1 and ADC-2 killed the tumor cells with higher maximum cytotoxicity%

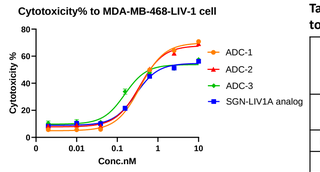

Table 2. The sensitivity of breast tumor cell lines to free payloads *in vitro*.

Cell lines	Maximum cytotoxicity% of free payloads	
	MMAE	Dxd
MDA-MB-468-LIV1	56.15 %	76.19 %
MCF7-LIV1	64.33 %	80.19 %
MCF7	52.1 %	62.7 %
T47D	38 %	64 %

Figure 5. *In vitro* cytotoxicity to MDA-MB-468-LIV-1 cell. The maximum cytotoxicity was significantly higher for Topo I inhibitor payload based ADCs than MMAE based ADCs in multiple breast cancer cell lines.

Topo I inhibitor based ADCs exhibited more potent anti-tumor activities *in vivo*

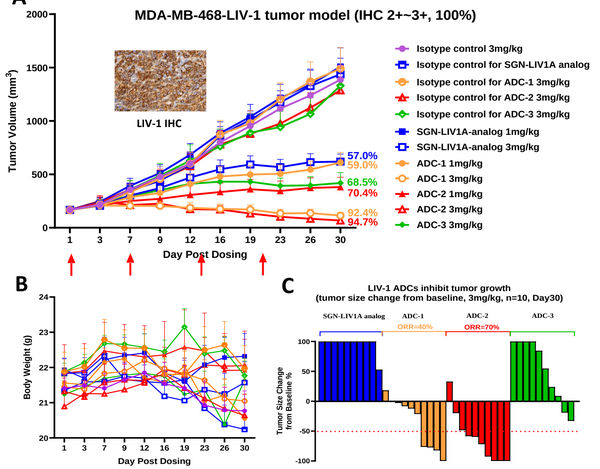

Figure 6. Efficacy of LIV-1 ADCs head-to-head comparison with SGN-LIV1A in MDA-MB-468-LIV-1 tumor model in BALB/c nude mice.
Mice were inoculated with 5*10^6 MDA-MB-468-LIV1 tumor cell per mouse and mixed with 50% Matrigel, when tumor size around 150-200 mm3, ADCs were i.v injected. A, Tumor growth curve. B, Body weight change of mice. C, Tumor size change of 3mg/kg groups.

ADC-1 and ADC-2 have strong bystander effect without ADCC activity *in vitro*

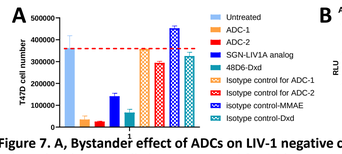

Figure 7. A, Bystander effect of ADCs on LIV-1 negative cells. MDA-MB-468-LIV-1 cells were co-cultured with T47D cells (LIV-1 negative) for 96 hrs. Ratio of MDA-MB-468-LIV-1: T47D=1:3. **B, ADCC activity on MDA-MB-468-LIV-1 cell measured by Jurkat-NFAT-Luc-FcγRIIIA-158V reporter assay.**

ADC-1 and ADC-2 displayed durable tumor regression activities at 6-10 mg/kg

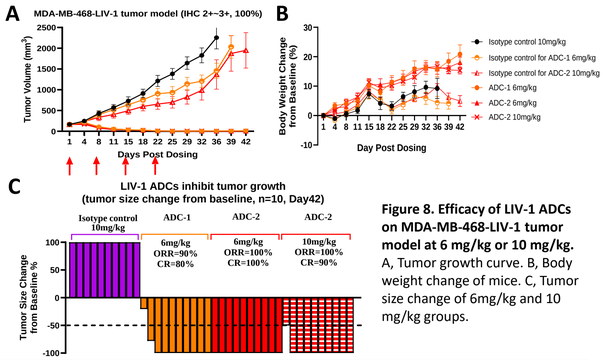

Figure 8. Efficacy of LIV-1 ADCs on MDA-MB-468-LIV-1 tumor model at 6 mg/kg or 10 mg/kg. A, Tumor growth curve. B, Body weight change of mice. C, Tumor size change of 6mg/kg and 10 mg/kg groups.

Conclusions

- 48D6 is a novel humanized anti-LIV1 antibody binding to a unique epitope.
- ADC-1 and ADC-2 are novel LIV-1 ADCs with 48D6 site-specifically conjugated with Topo I inhibitor payloads. With higher affinity and specificity, they can be internalized by the tumor cells and kill the tumor more efficiently.
- Although the cytotoxicity activity of ADC-1 and ADC-2 were similar to that of SGN-LIV1A analog *in vitro*, they exhibited much more potent tumor inhibition than SGN-LIV1A analog *in vivo and* both ADCs regressed the tumors completely.
- Both ADC-1 and ADC-2 have strong bystander effect to overcome tumor heterogeneity.
- By site-specific conjugation, ADCC activity and other Fc functions of antibody were depleted, which may reduce Fc mediated non-specific binding and cytotoxicity.
- As potential next-generation therapeutic agent for LIV-1 expressing solid tumors, ADC-1 and ADC-2 are being further developed for clinical testing.

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.
2. Rizzo, A., Cusmai, A., Acquafredda, S., Rinaldi, L., & Palmiotti, G. (2022). Ladiratuzumab vedotin for metastatic triple negative cancer: preliminary results, key challenges, and clinical potential. Expert Opinion on Investigational Drugs, 31(6), 495–498. https://doi.org/10.1080/13543784.2022.2042252

Contact
Email: Xueming.qian@transcenta.com

Abstract No: SESS-3612