QL535, A Novel CD2-Costimulating T Cell Engager Targeting PSMA-Positive Prostate Cancer Matching CD28 Trispecific Antibody in Cytotoxicity, Minimizing Cytokine Release and T-Cell Exhaustion

Xiao Liu[1], Shenda Gu[1], Ke Liu[2], Joshua Morrissette[1], Jiantao Kong[2], Guangjie Guo[1], Yuxia Wang[2], Xin Zhang[2], Jiaming Cao[2], Moyan Hu[2], Min Sun[2], Ruixue Ma[2], Jane Chen[1], Nathan Ngo[1], Hejie Zhu[2], Liwei Wang[2], Yan Fang[2], Hao Liu[2], Shuyong Zhao[2], Shihao Chen[1]

QLSF Biotherapeutics, Inc., South San Francisco, CA, USA | 2. QILU Pharmaceutical Co., Ltd., Jinan, China | 3. QILU Pharmaceutical Co., Ltd., Shanghai, China

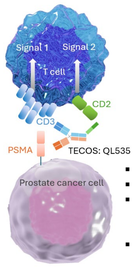
Abstract 3495 AACR 2025

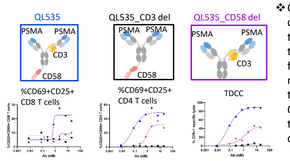
Introduction

Prostate cancer is a leading cause of cancer-related mortality in men, highlighting the need for new therapeutic options due to resistance to current treatments[1,2]. PSMA (Prostate-Specific Membrane Antigen) is overexpressed in prostate cancer tissues compared to normal prostatic tissue rendering it an ideal target for prostate cancer therapies, enabling precise delivery of therapeutic agents to cancer cells[3].

We developed QL535, a novel trispecific antibody in a 2+1+1 format designed to bridge tumor cells via PSMA and activate T cells through CD3 (signal 1) and CD2 (signal 2) via its ligand CD58. This design aims to stabilize the immune synapse and enhance T-cell cytotoxicity. Additionally, it overcomes T-cell exhaustion, with fine-tuned CD3 affinity to improve safety and reduce the risk of cytokine release syndrome (CRS).

Our TECOS 'T Cell Engager with Co-Stimulation' Platform with CD2-Costimulating

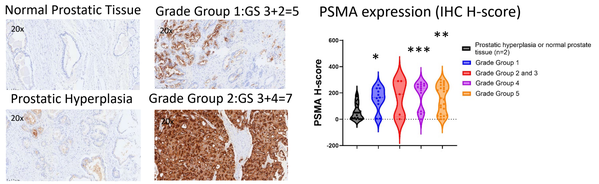




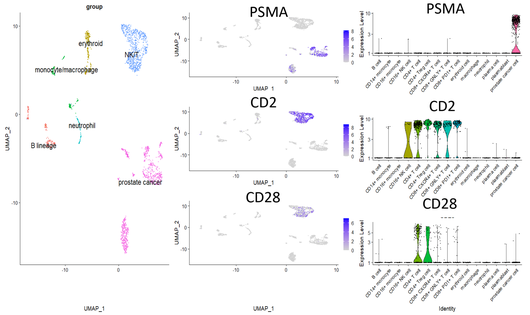
- QL535 is TECOS 'T Cell Engager with Co-Stimulation' Platform with CD2-Costimulating.
- Maintaining immune synapse function through direct provision of CD2 co-stimulation.
- CD2 Co-stimulation has been shown to enhance T cell adaptability, restore exhausted T cells (Tex), promote T-cell proliferation and overcome resistance to T cell therapy (TCE) in T cell-deficient solid tumor models.
- By reducing CD3 affinity, QL535 can improve tolerability, minimize antigen non-specific activation, avoid or reduce TMDD, and thereby clinically control safety.

❖ Comparative analysis was conducted with three trispecific antibody formats targeting CD28 and three formats targeting CD2, resulting in a lead CD2 trispecific antibody as QL535, alongside a CD28 trispecific antibody as a control.

Target Discovery and Evaluation



We evaluated PSMA expression in 77 Chinese prostate cancer patients using immunohistochemistry (IHC), confirming its expression in Chinese malignant prostate tissues.



Analysis of single-cell RNA sequencing data from 2,170 cells across 14 mCRPC patients revealed that CD2 expression on tumor-infiltrating lymphocytes (TILs), particularly CD8⁺ T cells, exceeds that of CD28, underscoring the potential for CD2 targeting in enhancing antitumor immunity.

QL535 Exhibits Favorable Drug Developability

1. QL535 exhibits similar CD2 and CD3 binding affinity to both human and cynomolgus by SPR analysis.

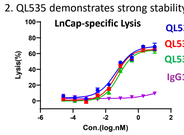
CD3_SPR

Specimen	ka (1/Ms)	kd (1/s)	K_D (M)
Recombinant Human CD3	3.36E+05	0.008552	2.54E-08
Recombinant Cynomolgus Monkey CD3	3.65E+05	0.008743	2.39E-08

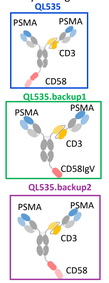
CD2_SPR

Specimen	ka (1/Ms)	kd (1/s)	K_D (M)
Recombinant Human CD2	1.59E+05	0.004589	2.88E-08
Recombinant Cynomolgus Monkey CD2	1.52E+05	0.004454	2.93E-08

2. QL535 demonstrates strong stability in human serum.



3. QL535 outperforms the other two formats in accelerated stability testing.



5mg/mL @ 42°C

Week	%Agg	%Main	%Frag
0	1.2	98.4	0.4
1	2.4	96.4	1.2
2	3.7	94.3	2.0
3	4.5	92.9	2.6
4	5.4	91.4	3.2

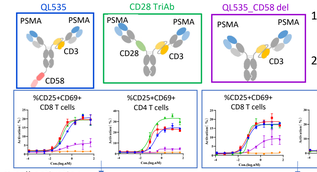
5mg/mL @ 42°C

Week	%Agg	%Main	%Frag
0	1.6	97.8	0.6
2	5.9	92.6	1.5
4	12.5	84.2	3.3

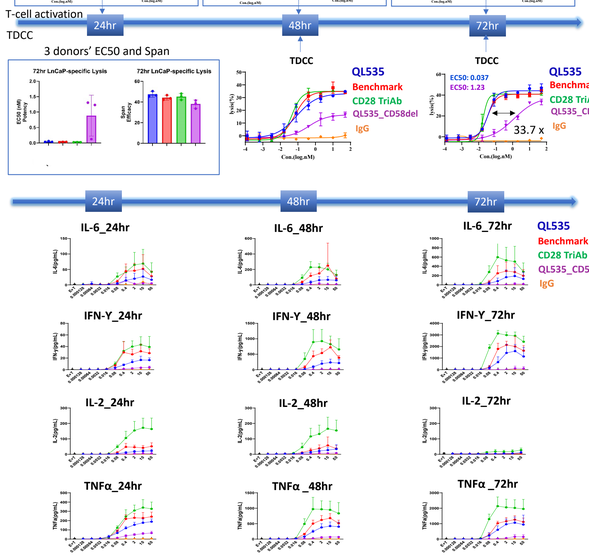
5mg/mL @ 42°C

Week	%Agg	%Main	%Frag
0	1.6	97.7	0.7
1	2.6	95.3	2.1
2	2.8	94.6	2.6
3	3.8	92.6	3.6
4	6.0	90.5	3.5

QL535 Achieves CD28 Tri-specific Antibody Cytotoxicity with Reduced Cytokine Release



1. Compared to bispecific antibodies, QL535 can significantly activate T cells specific to tumors *in vitro* and kill tumor cells (LnCaP) that highly express PSMA.
2. QL535 achieves the same level of cytotoxicity as the CD28 trispecific antibody and clinical benchmarks



QL535's cytokine release levels (IL-6, IFN-γ, IL-2, TNFa) are lower than those of clinical benchmark and CD28 trispecific antibody at the same level of cytotoxicity.

QL535 can Amplify the pPLC-γ1 Signaling Pathway to Facilitate T-Cell Activation

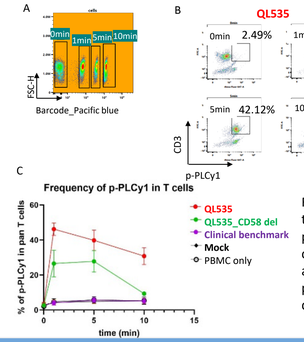
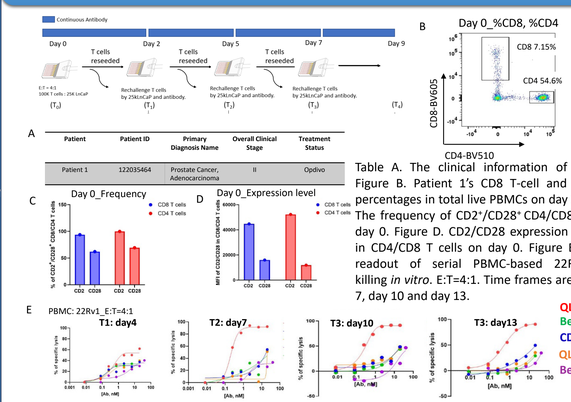


Figure A. The experiment used barcode technology to differentiate samples from four time points to prevent batch effects. Figure B. Representative flow cytometry data comparing QL535 and the bispecific antibody. Figure C. Dot-line graphs of the percentage of pPLCγ1+ T cells for three individual donors and the combined data.

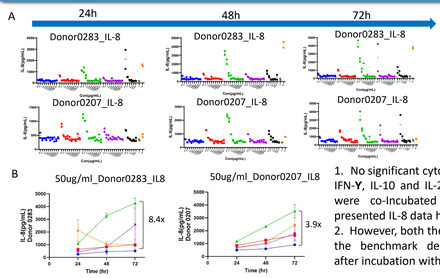
QL535 can Continuously Induce PBMCs in Prostate Cancer Patients Receiving Anti-PD1 Treatment to Sustain Cytotoxicity Against Tumor Cells *in vitro*.



Patient	Patient ID	Primary Diagnosis Name	Overall Clinical Stage	Treatment Status
Patient 1	122035464	Prostate Cancer, Adenocarcinoma	II	Opdivo

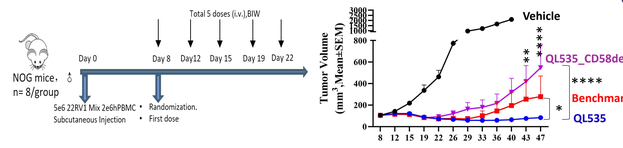
Table A. The clinical information of patient 1. Figure B. Patient 1's CD8 T-cell and CD4 T-cell percentages in total live PBMCs on day 0. Figure C. The frequency of $CD2^+/CD28^+$ CD4/CD8 T cells on day 0. Figure D. CD2/CD28 expression level (MFI) in CD4/CD8 T cells on day 0. Figure E. CellTilter readout of serial PBMC-based 22Rv1-specific killing *in vitro*. E:T=4:1. Time frames are day 4, day 7, day 10 and day 13.

No Significant Cytokine Release is Detected When PBMCs are Co-Incubated with QL535 *in vitro*



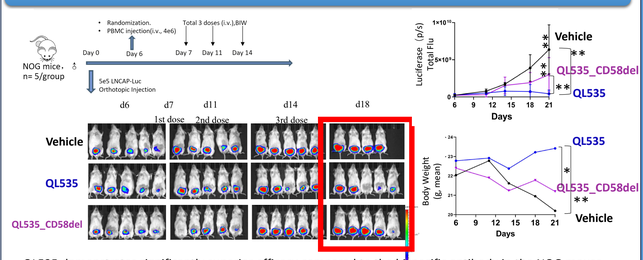
1. No significant cytokine release (IL-6, IL-8, TNFα, IFN-γ, IL-10 and IL-2) was detected when PBMCs were co-Incubated with QL535 *in vitro*. We presented IL-8 data here.
2. However, both the CD28 trispecific antibody and the benchmark demonstrated cytokine release after incubation with PBMCs.

Superior Efficacy of QL535 in Xenografted NOG Mouse Model with 22RV1 Tumors



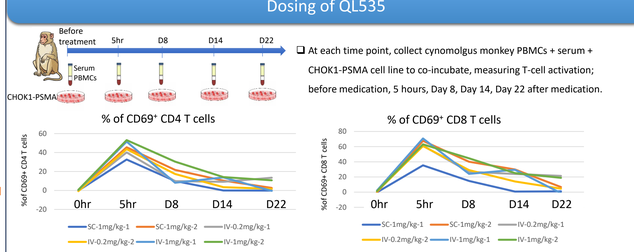
In the *in vivo* efficacy comparison study in a NOG mouse xenograft model of 22RV1 tumors (with medium to low PSMA expression), QL535 demonstrated superior efficacy compared to the bispecific antibodies and clinical benchmark.

Superior Efficacy of QL535 in LNCAP-Luc NOG Mouse Orthotopic Transplant Model



QL535 demonstrates significantly superior efficacy compared to the bispecific antibody in the NOG mouse orthotopic transplant model of LNCAP-Luc (high PSMA expression) human prostate cancer.

Activation of T Cells in PBMCs by Serum from Cynomolgus Monkeys After Single Dosing of QL535



❑ At each time point, collect cynomolgus monkey PBMCs + serum + CHOK1-PSMA cell line to co-incubate, measuring T-cell activation; before medication, 5 hours, Day 8, Day 14, Day 22 after medication.

Regardless of whether administered via IV or SC, after a single dose of QL535 in cynomolgus monkeys, T cells in PBMCs can be activated by serum, which were extracted at 5hr time point, with co-cultured PSMA-positive cell lines *in vitro*.

Conclusions

- **Target Specificity and Costimulation**: QL535, a TECOS with CD2 costimulation, specifically targets PSMA-positive prostate cancer, offering an advantageous approach where CD28 costimulatory factors are minimally expressed at the tumor site.
- **Therapeutic Developability**: QL535 demonstrates favorable therapeutic developability, indicating its potential for efficient and scalable production and clinical application.
- **Efficacy and Safety Profile**: QL535 achieves the same *in vitro* killing efficacy as the CD28 trispecific antibody, but with an improved safety profile characterized by reduced cytokine release.
- **Mechanism of Action**: The mechanism of action of QL535 involves the amplification of the pPLC-γ1 signaling pathway, enhancing its therapeutic effect.
- **Persistent Activation**: QL535 is capable of repeatedly stimulating prostate cancer patient PBMCs, enabling them to continuously target and eliminate PSMA+ cancer cells.
- ***In Vivo* Efficacy**: Demonstrated excellent efficacy in *in vivo* models, further validating QL535's potential as an effective treatment for prostate cancer.
- **Immune Activation**: Serum from cynomolgus monkeys following a single dose of QL535 can activate T-cells antigen-dependently *in vitro*, underscoring its potential to enhance immune responsiveness against tumors.

Disclosures

X Liu, S Gu, J Morrissette, G Guo, J Chen, N Ngo, S Chen are current or former employees of QLSF Biotherapeutics, Inc. receiving salaried compensation and stock options.
K Liu, J Kong, Y Wang, X Zhang, J Cao, M Hu, M Sun, R Ma, H Zhu, L Wang, Y Fang, H Liu, S Zhao are current employees of Qilu Pharmaceutical Co., Ltd. receiving salaried compensation.

Selected References

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71(3), 209–249.
2. American Cancer Society. (2025). *Key statistics for prostate cancer*.
3. Scherer, K., Kumar, A., & Lütje, S. (2020). "Prostate-Specific Membrane Antigen: Advancements in Targeted Therapy and Imaging Modalities." *Journal of Nuclear Medicine*, 61(1), 82-89.