QL301, a PD-L1 dependent 4-1BB agonist with minimal liver toxicity

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Background

4-1BB (CD137, TNFRSF9) is a potent co-stimulatory receptor found on T and NK cells[1]. Activation of 4-1BB requires receptor clustering, which is naturally mediated by the endogenous trimeric 4-1BB ligand[2]. Cross-linking via agonist monoclonal antibodies also activates 4-1BB, but can result in unwanted side effects, mainly liver toxicity[3]. To address the shortcomings of 4-1BB agonist antibodies, we developed QL301, a PD-L1 x 4-1BB bispecific antibody that conditionally activates 4-1BB only when engaging PD-L1, an immune checkpoint receptor ligand, elevated in the immuno-suppressive tumor microenvironment.

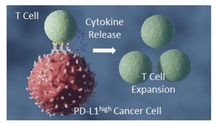
Design Parameters

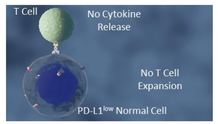
QL301 is built on a silenced human IgG1 backbone, eliminating Fc gamma receptor functions such as antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). QL301 was constructed from antibodies generated at QLSF, a 4-1BB scFv attached to the C-terminus of our novel anti-PD-L1 antibody. In a 2 x 2 configuration, QL301 cross-links 4-1BB on T cells upon binding to APC or tumor cells expressing PD-L1.

PD-L1 Binding
- Promotes tumor targeting and accumulation
- Blocks the PD-1 / PD-L1 checkpoint pathway
- Drives PD-L1 dependent activation of 4-1BB

Inert Human Fc
- No FcγR binding, 4-1BB activation is PD-L1 binding dependent
- No ADCC or CDC, no depletion of effector cells

4-1BB Binding
- Provides potent co-stimulation of T cells
- No 4-1BB activation without PD-L1 binding



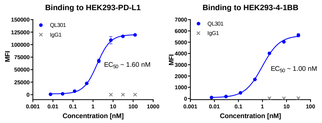


Target Validation

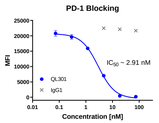
QL301 has a 40-fold higher affinity for PD-L1 over 4-1BB, designed for enhanced tumor accumulation. QL301 binds to HEK293 cells expressing PD-L1 or 4-1BB.

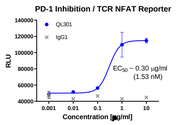
Binding Affinity by SPR

	PD-L1	4-1BB
Human	0.349 nM	13.4 nM
Cynomolgus	0.355 nM	16.8 nM

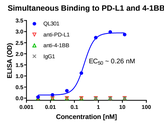


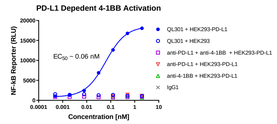
QL301 blocks PD-1 and restores TCR signaling in a NFAT reporter assay.





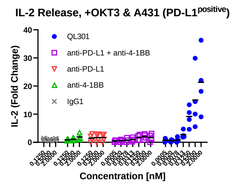
QL301 simultaneously bound to both PD-L1 and 4-1BB in a sandwich ELISA. In a NF-kB reporter assay, QL301 activated 4-1BB only in the presence of HEK293 expressing PD-L1.

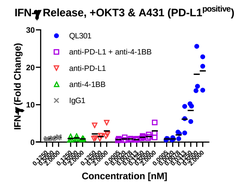




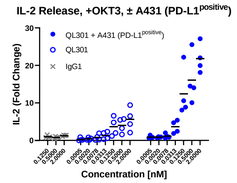
In Vitro Activity

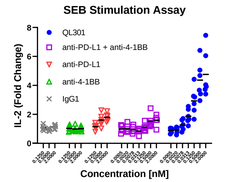
Human PBMC were stimulated with anti-CD3 (OKT3) and incubated with QL301, anti-PD-L1 or anti-4-1BB monoclonal antibodies or their combination. In the presence of PD-L1^{positive} A431 cells, QL301 induced IL-2 and IFNγ release while anti-PD-L1 or 4-1BB antibodies alone or in combination did not.



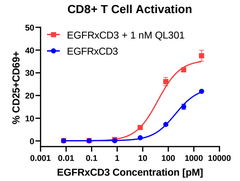


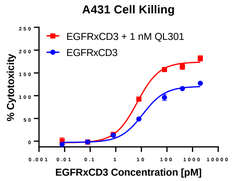
The presence of PD-L1^{positive} A431 cells results in enhanced IL-2 induction. Enhanced IL-2 expression was also observed in an SEB stimulation assay in the presence of QL301, but not with anti-PD-L1 or 4-1BB monoclonals or their combination.





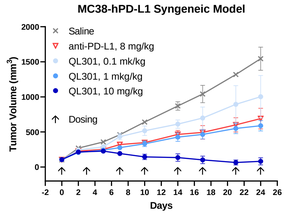
QL301 augmented the potency of an EGFRxCD3 bispecific antibody. The combination with QL301 activated more T cells and enhanced the killing of EGFR^{high}/PD-L1^{positive} A431 cells in vitro.

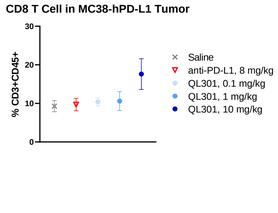




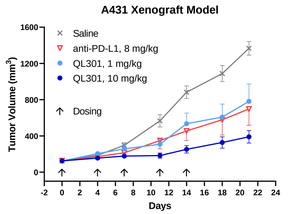
In Vivo Efficacy

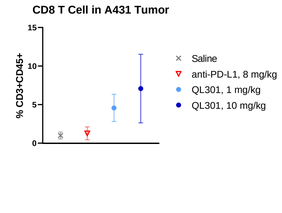
QL301 suppressed the growth of MC38 murine colon adenocarcinoma cells expressing human PD-L1 in a human PD-L1 and 4-1BB double knock-in C57BL/6 mouse model. QL301 at 10 mg/kg was significantly more efficacious than the QLSF's PD-L1 monoclonal antibody at the equal molar dose of 8 mg/kg ($p < 0.0001$, n = 6). Analysis of tumor infiltrating immune cells at the end of study showed more CD8 T cells in the tumors of animals that received QL301 compared to saline or anti-PD-L1 monoclonal treatment ($p < 0.01$).





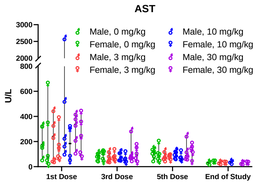
QL301 suppressed the growth A431 human cancer cells co-implanted with human PBMC in CB17-SCID mice. QL301 has better tumor growth inhibitory effect than the QLSF PD-L1 monoclonal antibody with higher percentage of CD8 T cells in the tumor (n = 8).

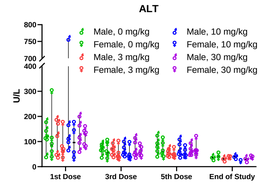




NHP Toxicology

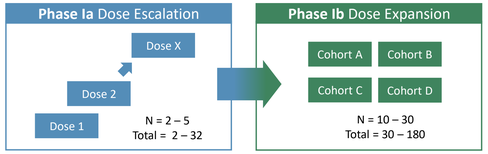
QL301 was well tolerated in a repeated dose toxicology study in rhesus monkeys, without inducing chronic elevation of AST and ALT.





Phase 1 Clinical Study

- Ongoing. The first patient was dosed in November of 2021. NCT05150405.
- Objectives: safety, tolerability, and early efficacy
- Format: i3 + 3, Q2W IV infusion, DLT period = 28 days
- Indications: locally advanced, recurrent or metastatic solid tumors, or hematological tumors refractory to standard of care



Summary

- QL301 is a bispecific antibody that blocks PD-L1 and activates 4-1BB.
- The silenced IgG1 backbone eliminates FcγR binding and therefore prevents non-specific 4-1BB activation, ADCC, and CDC.
- Activation of 4-1BB signaling via receptor cross-linking is restricted to the presence of PD-L1^{positive} cells.
- QL301 has robust anti-tumor efficacy in vivo.
- QL301 was well tolerated in rhesus monkeys.
- A phase 1 clinical trial is ongoing.

Disclosures

S Gu, WW Prior, I Tang, HV Tran, A Chan, A McClain, A Kurtzman, and S Chen are current or former employees of QLSF Biotherapeutics, Inc. receiving salaried compensation and stock options.

Y Luo, X Kang, X Wu, Q Zheng, G Jia are current employees of QILU Pharmaceutical Co., Ltd., receiving salaried compensation.

References

1. Kim HH, Kwack K, Lee ZH. Mol Cells. 2000;10(3):247-252.
2. Won EY, Cha K, Byun JS, et al. J Biol Chem. 2010;285(12):9202-9210.
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