QL615, a tetraspecific T cell engager with highly restricted activity on MET and EGFR-positive tumors while providing co-stimulation via CD2

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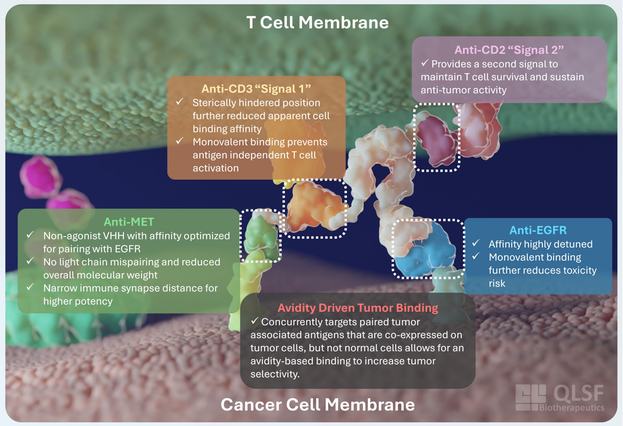
Background

Effective targeting of solid tumors using T cell engagers remains challenging due to the lack of tumor specific antigens (TSA), and the difficulty in achieving sustained anti-tumor response. Frequently, target antigen expression is upregulated on cancer, yet low expression on normal tissues limits the therapeutic window. Moreover, the lack of co-stimulatory signals paired with CD3 in the tumor microenvironment often leads to T cell exhaustion and non-durable responses.

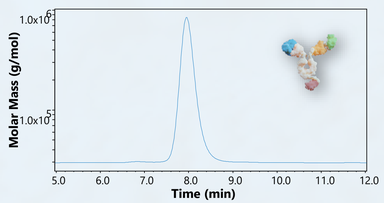
To overcome these shortcomings, we have developed a tetraspecific T cell engaging platform, termed **TECAD** "**T** Cell **E**ngager with **C**o-Stimulation **A**vidity **D**riven", to address both tumor selectivity and potency by 1) activating T cells only when bound to tumor cells expressing paired antigens, and 2) providing CD3 co-stimulation via conditional CD2 activation. The latter enhances antitumor immune response and overcomes resistance to immune checkpoint blockade therapies.

Key Design Features

- EGFR binding affinity is highly reduced and is configured opposite of the CD3 arm to minimize EGFR-specific toxicity.
- MET binding is mediated a non-agonist VHH with affinity optimized for pairing with the affinity optimized EGFR binder.
- The combination of EGFR Fab and MET VHH ensures that there is no light chain mispairing, resulting in an antibody with molecular weight similar to that of native IgG, and allows for optimal immune synapse distance between tumor and T cells.
- Sterically hindered and monovalent CD3 binding ensures MET and EGFR dependent T cell activation and reduced risk for cytokine release syndrome.
- CD2 agonist provides a co-stimulation for invigorating and sustaining T cell response and contains proprietary mutations for improved stability and ease of manufacturing.

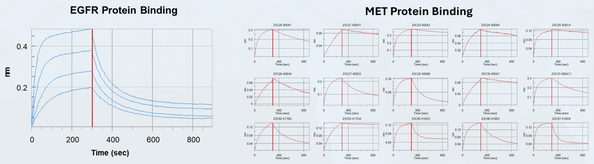


Over 99% monomer after 2-step purification

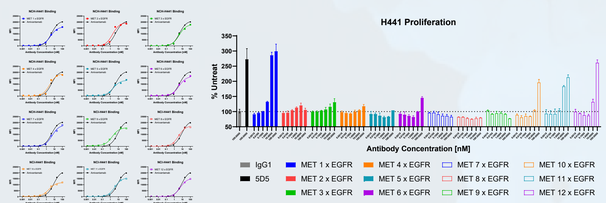


Antibody Discovery

The affinity of the EGFR binding arm was significantly reduced from that of the parental clone to double digit nanomolar range to minimize EGFR-specific toxicity. A panel of VHH clones with a wide range of binding affinities to MET was obtained from alpaca immunization.



Optimal MET/EGFR pairs were non-agonists but retained strong avidity binding to MET^{pos}/EGFR^{pos} cells

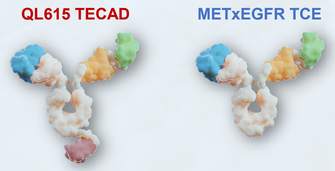


MET VHH clones were paired with the affinity reduced anti-EGFR clone to generate MET/EGFR bispecific antibodies and tested for binding to NCI-H441, which expresses both MET and EGFR. To select for MET/EGFR pairs which will not activate MET signaling and cell proliferation, a proliferation assay was setup using H441 cells. The bivalent MET antibody 5D5 from onartuzumab was used a positive control. MET/EGFR pairs with binding affinity comparable to amivantamab but did not induce H441 proliferation were selected for further evaluation.

Generation of Lead Candidate

Anti-MET VHH clones were humanized by grafting the CDRs onto the closest human framework and backmutations were introduced to minimize impact on binding affinity. Tetraspecific antibodies were built with different configurations, including N- and C-terminal fusion of a CD2 agonist, on the same or opposite arm as CD3.

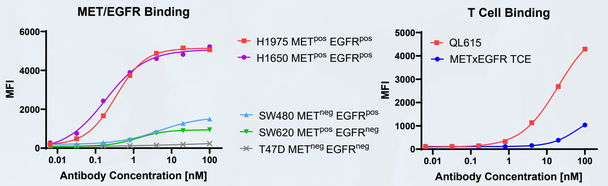
The lead candidate incorporates a CD2 agonist at the c-terminus. A trispecific METxEGFR TCE lacking the CD2 co-stimulation domain was built and used throughout preclinical evaluation for comparison.



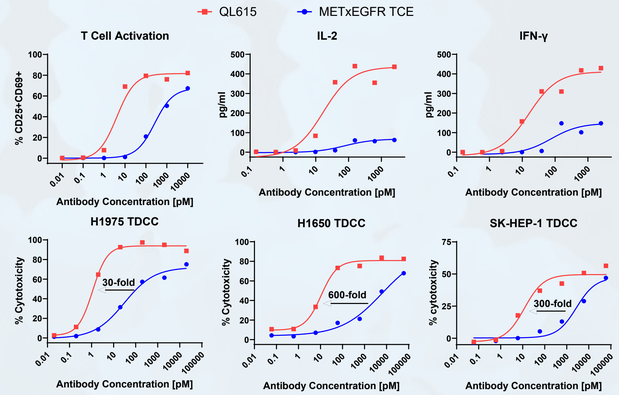
In Vitro Validation

The binding of QL615 was evaluated across cancer cell lines expressing varying levels of MET and EGFR. In the example shown below, QL615 showed strong avidity-driven binding to H1975 and H1650, which express both EGFR and MET, significantly less binding to SW620 and SW480 expressing one of the two receptors, and no binding to T47D lacking either receptor.

QL615 containing the CD2 binding domain demonstrated dose-dependent binding to T cells, superior to the METxEGFR TCE control, suggesting enhanced T-cell recruitment capabilities.

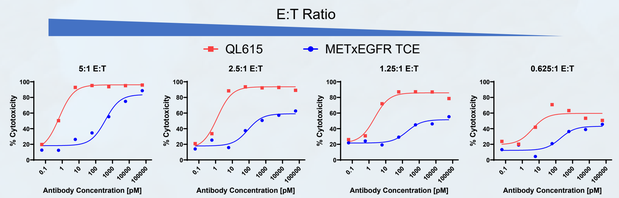


QL615 had 30 to 600-fold improvement in TDCC potency over the METxEGFR TCE without co-stimulation



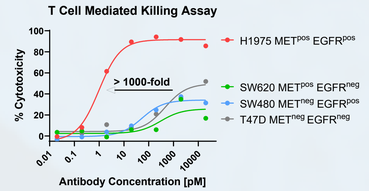
The incorporation of a CD2 agonist led to more potent T cell activation, cytokine release, and 30 to 600-fold improvement over the METxEGFR TCE without co-stimulation in mediating killing of the MET^{pos}EGFR^{pos} cancer cells.

QL615 maintained potency at low E:T ratio



QL615 consistently showed superior cytotoxicity and maintained potency at low E:T ratio, further highlighting the role of CD2 co-stimulation in enhancing T-cell recruitment and anti-tumor activity.

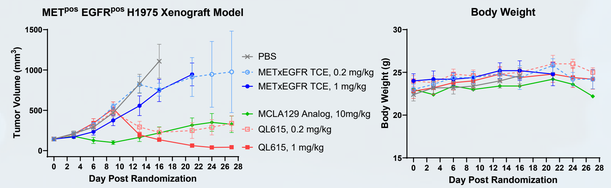
QL615 maintained high selectivity towards tumor cells co-expressing MET and EGFR



Robust cytotoxicity was observed against MET and EGFR double positive cancer cell lines, but only moderate cytotoxic activity against MET and EGFR single positive colon cancer cells SW620 and SW480, and the double negative T47D breast cancer cell line, supporting the avidity driven specificity of QL615 TECAD.

In Vivo Efficacy

Treatment with QL615 resulted in dose-dependent growth inhibition of the H1975 tumor in NCG mice reconstituted with human PBMC. QL615 demonstrated superior anti-tumor efficacy compared to the METxEGFR TCE without CD2 co-stimulation. In addition, QL615 achieved significant tumor reduction by day 27, whereas tumor size had rebound after treatment with an analog of MCLA129. No significant body weight loss was observed across treatment groups, suggesting acceptable tolerability of the tested constructs.



Summary

- QL615, our lead TECAD candidate, targets MET and EGFR, two clinically validated targets with limited co-expression on normal tissues.
- QL615 showed nanomolar EC50 on tumor cells expressing both MET and EGFR, whereas binding was negligible on cells expressing only low levels of either MET or EGFR.
- In vitro killing assays demonstrated over a 1000-fold selectivity in the lysis of double-positive tumor cells. The TECAD's CD2-mediated co-stimulation enhanced in vitro killing of tumor cells by 30 to 600-fold over constructs with CD3 redirection alone.
- QL615 showed superior tumor growth control in vivo.
- QL615 is ready for further development and clinical evaluation.

Disclosures

All authors are current or former employees of QLSF Biotherapeutics, Inc. receiving salaried compensation and stock options.

Selected References

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Partnering

- QLSF Biotherapeutics is open to co-development, strategic partnerships or out-licensing opportunities to fully leverage our platform and programs.
- For additional information on QLSF programs please contact partnering@qlsfbio.com.