ZW209, a DLL3 targeted trispecific T cell engager with integrated CD28 co-stimulation, demonstrates safety and potent preclinical efficacy in models of small cell lung cancer

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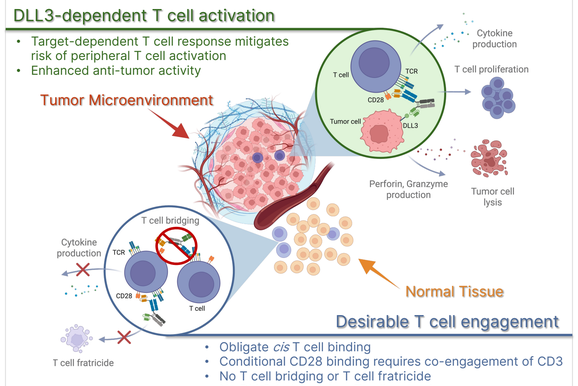
Abstract #7318

Introduction

Small cell lung cancer (SCLC) is a highly aggressive and difficult-to-treat malignancy with limited treatment options[1]. Delta-like ligand 3 (DLL3), a cell surface protein overexpressed in SCLC and other neuroendocrine carcinomas, has emerged as a promising therapeutic target[2,4]. Bispecific T cell engagers (TCE) targeting DLL3, including Imdelltra® (tarlatamab; AMG 757) which has received accelerated approval, have demonstrated anti-tumor activity in the clinic[5]. However, clinical activity of bispecific TCEs may be limited by low T cell infiltration and poor T cell function, highlighting an opportunity to improve the rate and depth of response[3].

Zymeworks' development candidate, ZW209, is a trispecific TCE designed to incorporate CD28 co-stimulation to improve durability of T cell mediated responses. ZW209 is designed to optimally engage CD3 and CD28 in an obligate *cis* manner, supported by a lack of T cell bridging and fratricide. Conditional CD28 engagement enhances DLL3-dependent cytokine induction and T cell proliferation with improved antitumor activity relative to clinical TCE benchmarks. Importantly, ZW209 displayed a favorable safety and PK profile in cynomolgus monkey study.

ZW209 is Designed for Optimized T cell Binding, Activation and Anti-tumor Activity



Design Facilitates Desirable T Cell Engagement

Exhibits obligate *cis* binding requiring co-engagement of CD3 to bind CD28

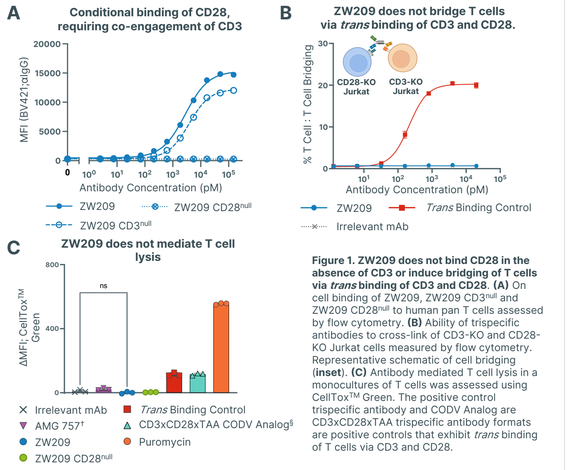


Figure 1. ZW209 does not bind CD28 in the absence of CD3 or induce bridging of T cells via *trans* binding of CD3 and CD28. (A) On cell binding of ZW209, ZW209 CD3null and ZW209 CD28null to human pan T cells assessed by flow cytometry. **(B)** Ability of trispecific antibodies to cross-link of CD3-KO and CD28-KO Jurkat cells measured by flow cytometry. Representative schematic of cell bridging (**inset**). **(C)** Antibody mediated T cell lysis in a monocultures of T cells was assessed using CellTox™ Green. The positive control trispecific antibody and CODV Analog are CD3xCD28xTAA trispecific antibody formats are positive controls that exhibit *trans* binding of T cells via CD3 and CD28.

ZW209 Mediates Sustained T Cell Activity

Sustained T cell-mediated cytotoxicity over repeated stimulations

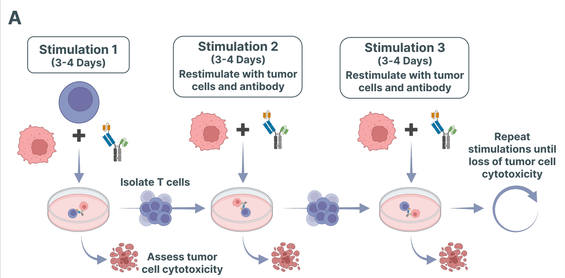


Figure 2. ZW209 Displays sustained T cell fitness while expanding memory T cell populations. (A) T cells were incubated with DLL3+ NCI-H82 cells and test article. For each subsequent round of stimulation, T cells were collected, counted, and re-stimulated with fresh NCI-H82 target cells and test article. Schematic of T cell restimulation. **(B)** Following each round of stimulation, co-cultures were assessed for tumor cell cytotoxicity. Following 3rd stimulation, ZW209 CD28null and AMG 757† showed no anti-tumor activity. **(C)** 3 days after 2nd stimulation (day 7), T cell memory populations were assessed by flow cytometry staining for CD45RO and CCR7 expression. T cells stimulated by ZW209 displayed an increased number of effector and central memory T cells relative to bispecific TCEs.

Enhanced DLL3-dependent T cell proliferation and survival

Figure 3. ZW209 Increases T cell proliferation and upregulation of anti-apoptotic marker Bcl-xL. (A) Test articles (5 nM) were incubated with CellTrace Violet™ labeled T cells alone or co-cultured with NCI-H82 cells for 5 days and assessed by flow cytometry. **(B)** Test articles (5 nM) were incubated with T cells co-cultured with NCI-H82 cells for 48 hours and evaluated for Bcl-xL expression by flow cytometry. ** p<0.01, *** p<0.001

Superior *In vitro* DLL3+ Tumor Cell Cytolysis

Improved potency relative to bispecific and trispecific clinical TCE benchmarks at low effector: target ratios

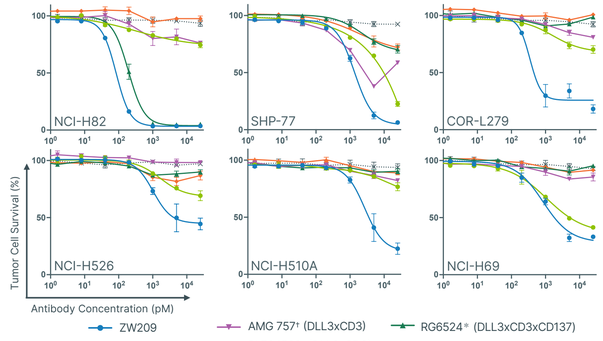


Figure 4. ZW209 displays superior cytotoxicity relative to bispecific and trispecific clinical TCE benchmarks across multiple DLL3-positive SCLC tumor cell lines. Test articles were incubated with T cells co-cultured with DLL3-expressing SCLC tumor cell lines at low E:T ratio for 7 days and evaluated for cytotoxicity.

ZW209 Mediates Enhanced *In vivo* Anti-tumor Activity

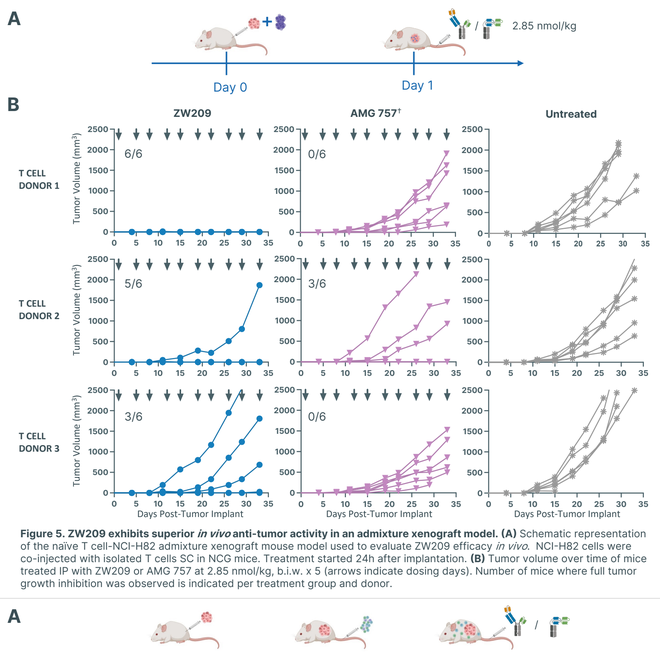


Figure 5. ZW209 exhibits superior *in vivo* anti-tumor activity in an admixture xenograft model. (A) Schematic representation of the naïve T cell-NCI-H82 admixture xenograft mouse model used to evaluate ZW209 efficacy *in vivo*. NCI-H82 cells were co-injected with isolated T cells SC in NCG mice. Treatment started 24h after implantation. **(B)** Tumor volume over time of mice treated IP with ZW209 or AMG 757 at 2.85 nmol/kg, b.i.w. x 5 (arrows indicate dosing days). Number of mice where full tumor growth inhibition was observed is indicated per treatment group and donor.

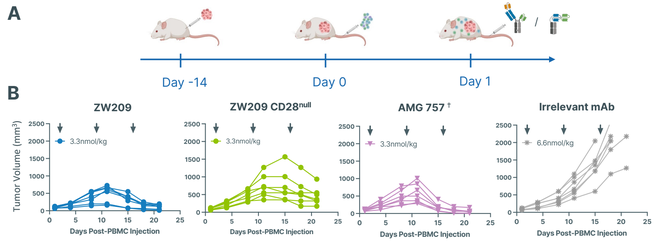


Figure 6. ZW209 displays *in vivo* anti-tumor activity in a humanized SHP-77 xenograft model. (A) Schematic of the naïve PBMC-engrafted SHP-77 xenograft mouse model used to evaluate ZW209 efficacy *in vivo*. SHP-77 cells were injected s.c. in NCG mice. Following PBMC humanization, mice were treated IV with test article q1w x 3. **(B)** Tumor volume over time of mice treated with ZW209 (3.3 nmol/kg), bispecific control (3.3 nmol/kg), AMG 757 (3.3 nmol/kg) and irrelevant mAb (6.6 nmol/kg). Full regression was observed in 6/7 mice treated with ZW209 and AMG 757. Arrows indicate treatment days.

ZW209 Displays Favorable *In vivo* Safety Profile

No systemic cytokine induction observed in an *in vivo* cytokine release model

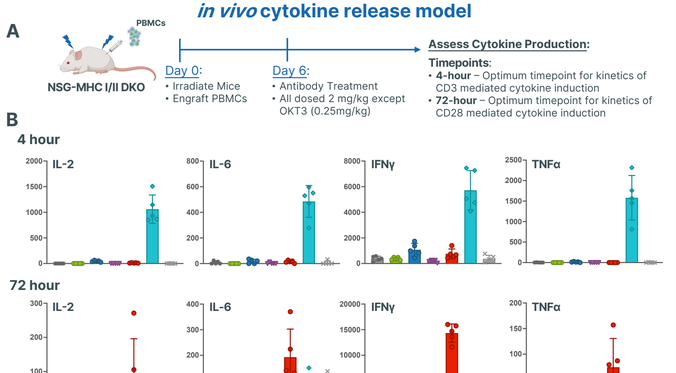


Figure 7. Humanized *in vivo* model for cytokine release. (A) Schematic of huPBMC engrafted *in vivo* model. **(B)** Mice were assessed for systemic cytokine production at 4 hours and 72 hours post-treatment.

Well-tolerated in Cynomolgus monkeys

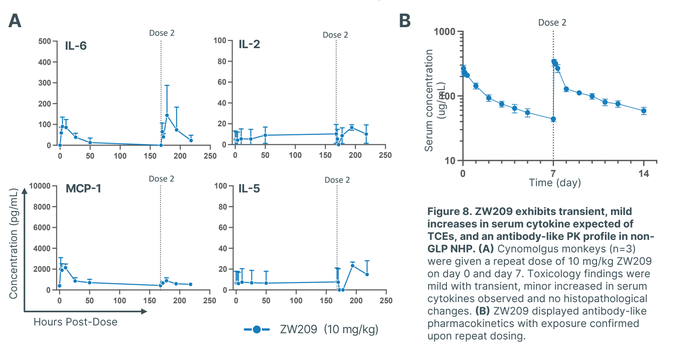


Figure 8. ZW209 exhibits transient, mild increases in serum cytokine expected of TCEs, and an antibody-like PK profile in non-GLP NHP. (A) Cynomolgus monkeys (n=3) were given a repeat dose of 10 mg/kg ZW209 on day 0 and day 7. Toxicology findings were mild with transient, minor increased in serum cytokines observed and no histopathological changes. **(B)** ZW209 displayed antibody-like pharmacokinetics with exposure confirmed upon repeat dosing.

Conclusions

ZW209, a DLL3 targeting trispecific T cell engager designed to optimally bind CD3 and CD28, was engineered using Zymeworks' TriTCE Co-Stim platform in combination with our Azymetric™ and EFECT™ technologies.

ZW209 has been engineered to promote:

- Enhanced anti-tumor activity compared to bispecific TCEs
- Enhanced T cell proliferation and survival
  - Prolonged and increased cytotoxicity over repeated tumor cell challenges
- Optimal T cell binding
  - Obligate *cis* T cell binding
  - Conditional CD28 engagement
  - No T-T bridging
  - No target-independent T cell activation
- Favorable tolerability and PK in non-human primate study

ZW209 has the potential to increase the depth and durability of responses in DLL3-expressing tumors by increasing T cell responses, which may translate to improved clinical outcomes

References:
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4. Yao, J. *et al.* 2022. DLL3 as an emerging target for the treatment of neuroendocrine neoplasms. Oncologist.
5. Myung-Ju, A. *et al.* 2023. Tarlatamab for Patients with Previously Treated Small-Cell Lung Cancer. N Eng J Med. 389:2063-2075

† AMG 757 (DLL3/CD3 BITE) produced in-house, ‡Bi 764532 (DLL3/CD3 bispecific TCE) produced in-house, * RG6524 (DLL3/CD2/CD137 trispecific TCE), § CD3xCD28xTAA CODV Analog is a CD3xCD28xMSLN trispecific with the same format as the Sanofi Trispecific containing a CD3xCD28 CODV-Fab; produced in-house. ◊ TGN1412 (hIgG4; biosimilar produced in-house)
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