ZW171, a differentiated 2+1 T cell-engaging bispecific antibody with antitumor activity in a range of mesothelin-expressing cancers

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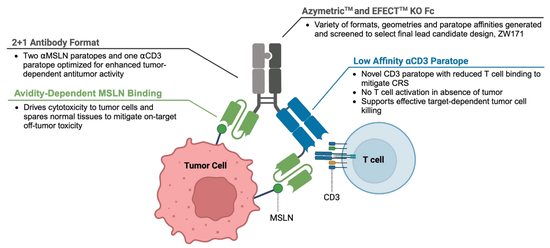
Abstract #3503

Introduction

Mesothelin (MSLN) is a tumor associated antigen overexpressed in many cancer indications and is an attractive target for immunotherapies including bispecific T cell engagers (TCE) and chimeric antigen receptor T (CART) cells. While MSLN-targeting immunotherapies have shown signs of clinical activity, their success has been hindered by dose-limiting toxicities associated with on-target off-tumor effects and cytokine release syndrome (CRS). To overcome these issues, we engineered ZW171, a MSLN-targeting TCE, with enhanced safety and anti-tumor activity.



Here, we assessed ZW171 activity in expanded MSLN-positive indications, benchmarking to other MSLN-targeting TCE, and in advanced patient-derived organoid and xenograft models. Additionally, the anti-tumor activity of ZW171 in the presence of shed MSLN (sMSLN) was assessed.

ZW171 Exhibits Activity Across a Range of MSLN-Expressing Indications

ZW171 Activity Correlates with MSLN Expression Levels

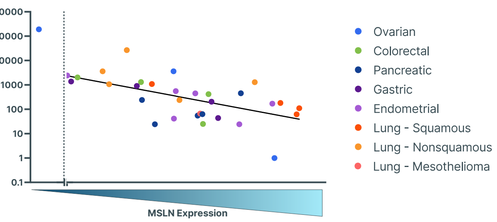
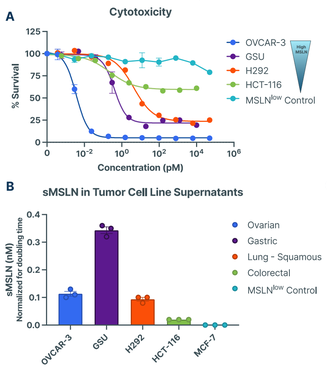


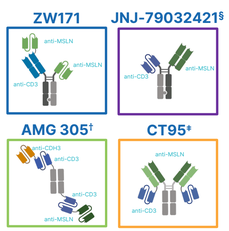
Figure 1. ZW171 induces T-cell mediated cytotoxicity in MSLN-expressing cells across multiple cancer indications. MSLN expression was quantified by flow cytometry using Quantum Simply Cellular anti-human IgG quantification beads (Bangs Laboratories). Test articles were incubated with human PBMC (Peripheral Blood Mononuclear Cells) co-cultured with MSLN-expressing cell lines for 3 days and evaluated for cytotoxicity. Potency fold change= cancer cell line IC_{50}/NIH:OVCAR-3 IC_{50}. Data consists of at least two human PBMC donors and two experimental repeats per cell line. Dotted line indicates expression level at which ZW171 exhibits limited cytotoxic activity. GraphPad Prism was used for Non-linear regression analysis and two-tailed Spearman correlation (Correlation p=***).



- ZW171 potency correlates with cell surface MSLN expression
- MSLN-expressing cell lines shed MSLN into supernatants
- ZW171 potency does not correlate with the concentration of MSLN shed by tumor cell lines

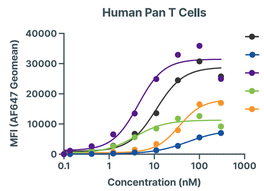
Figure 2. ZW171 maintains activity in the presence of shed MSLN. (A) Representative T cell-dependent cellular cytotoxicity (TDCC) data with ZW171 (mean ± SEM). Human PBMCs were co-cultured for 72 hr with cell lines that shed MSLN. **(B)** Measured shed MSLN from MSLN-expressing tumor cell lines of various cancer indications. Cell lines were cultured for 72 hrs and harvested supernatant was tested using a Human MSLN ELISA kit (R&D Systems). sMSLN data is an average of 3 experimental repeats (mean ± SEM), normalized for cell line doubling time. **(C)** Correlation analysis of ZW171 activity **(Fig 1)** compared to concentration of MSLN shed in tumor cell line supernatants **(B)**. Two-tailed Spearman correlation (Correlation p=0.13) analysis was completed using GraphPad Prism.

ZW171 Exhibits Reduced T Cell Binding and Increased Cytotoxic Activity Relative to Other Next Generation MSLN Targeting TCE



A

ZW171 Exhibits Reduced T Cell Binding



Test Article	Apparent Kd (nM)	Fold Reduction T Cell Binding* (95% Confidence Interval)
1+1 Gen 1 SP34	11	1.0
ZW171	56	6.4 (± 0.3)
JNJ-79032421	4.4	0.71 (± 0.3)
AMG 305	3.8	2.0 (± 0.7)
CT95 (LNK101)	36	2.4 (± 0.7)

*Fold reduction relative to 1+1 Gen 1 SP34 TCE benchmark

B

Test Article	Affinity by SPR Kd (nM)
ZW171	1.9 ± 0.05
AMG 305	23 ± 3.7
CT95 (LNK101)	117 ± 12
JNJ-79032421	ND*

*ND: not determined

ZW171 Exhibits Selective Tumor Cell Binding

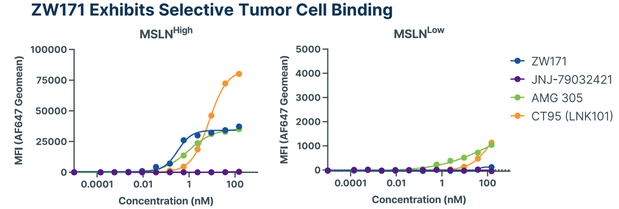


Figure 3. ZW171 binds with low affinity to CD3 and high affinity to MSLN. Test articles pre-labelled with Fab Gt anti Human IgG Fc AF647 were titrated and incubated with human pan-T cells **(A)** or tumor cells **(B)** for 1 hr, stained with live/dead stain, and assessed by flow cytometry. Results are background tracted using values obtained for irrelevant antibodies. **(A)** Table depicts Apparent Kd (nM) and AUC (Area under curve) fold reduction v.s. high-affinity 1+1 SP34 for each T-cell engager. **(B)** Table depicts MSLN affinity Kd (nM) determined by SPR (Surface Plasmon Resonance). †AMG 305 (MSLN/CD3/CDH3 BiTE), *CT95 (MSLN/CD3 bsAb), and §JNJ-79032421 (MSLN/CD3 bsAb) produced in-house. BiTE: Bi-specific T cell Engager. BsAb: Bispecific Antibody.

ZW171 Exhibits Potent Cytotoxicity In High-Mid But Not Low MSLN-Expressing Cells

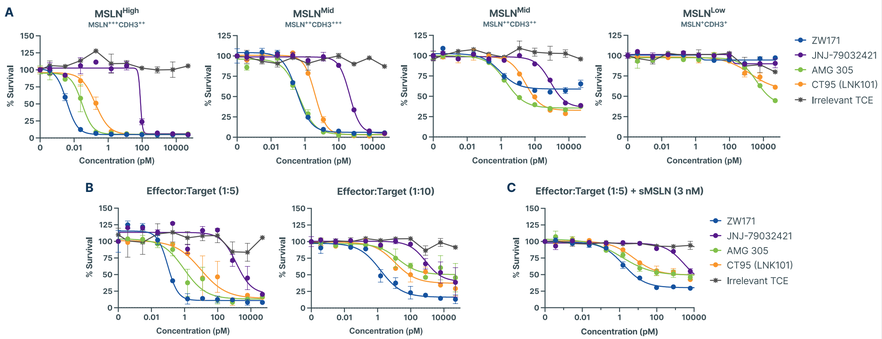


Figure 4. ZW171 displays equivalent or superior *In vitro* cytotoxic activity relative to competitor benchmarks at high and low effector to target ratios. (A) At a 5:1 effector to target (E:T) ratio, test articles were incubated with human PBMC co-cultured with a panel of MSLN and CDH3 expressing cell lines (NIH:OVCAR-3, NCI-H292, HCT-116, OVTOKO) for 72 hr and evaluated for cytotoxicity. **(B)** Test articles were incubated with human PBMC co-cultured with NIH:OVCAR-3 tumor cells at low E:T ratios for 120 hr and evaluated for cytotoxicity. **(C)** Test articles were incubated with human PBMC co-cultured with NIH:OVCAR-3 tumor cells (1:5 E:T ratio) in the presence of 3 nM soluble MSLN for 120 hr and evaluated for cytotoxicity. 3 nM of soluble MSLN was selected based on the highest level of shed MSLN observed in the serum of patients[5]. Data is an average of two individual donors (± SEM).

ZW171 Induces MSLN-Dependent T cell Activation and Cytokine Release

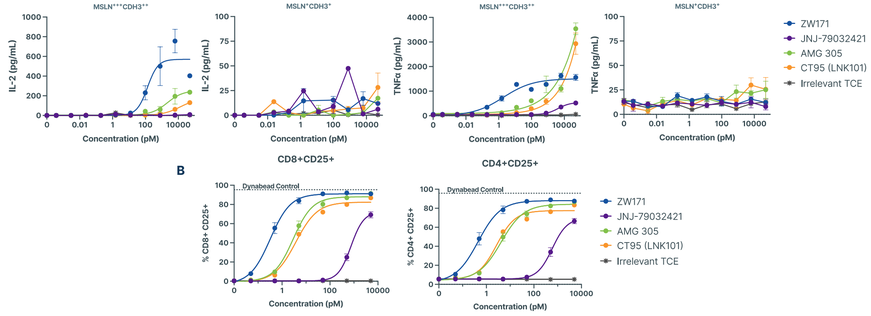
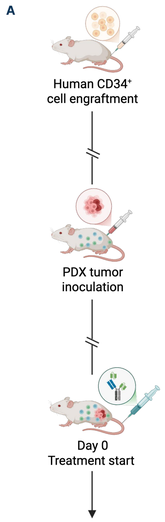


Figure 5. ZW171 induces cytokine production and T cell activation in cocultures of T cells and MSLN overexpressing tumor cells. (A) Test articles were incubated with human PBMC co-cultured with NIH:OVCAR-3 or OVTOKO tumor cells. After 72 hr, supernatants were harvested and evaluated for IL-2 and TNFα production by MSD, presented as mean ± SEM. **(B)** Test articles were incubated with human pan-T cells with NIH:OVCAR-3 tumor cells for 72 hr and evaluated by flow cytometry for %CD4+CD25+ and %CD8+CD25+ as a measure of T cell activation. Data is an average of 3 experimental repeats from two individual donors (± SEM).

ZW171 Exhibits Anti-Tumor Activity in Established PDX *in vivo* Models of NSCLC and PDAC Cancer



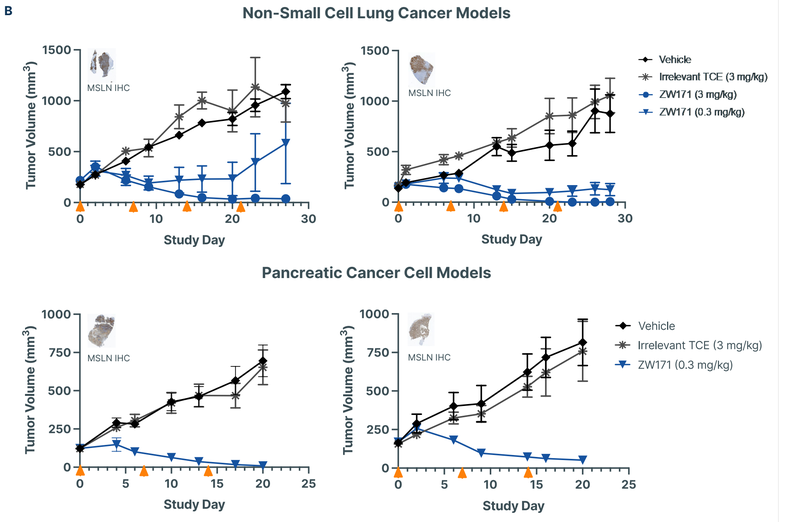
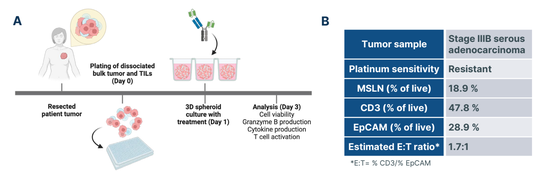


Figure 6. ZW171 exhibits potent anti-tumor activity in PDX models of non-small cell lung and pancreatic cancer. A) Schematic of study workflow. **B)** ZW171 mediated tumor growth inhibition in two NSCLC (Non-small cell lung cancer, top) and two PDAC (Pancreatic ductal adenocarcinoma, bottom) PDX (patient-derived xenograft) models. CD34+ humanized NCG were engrafted with patient-derived tumors. Once tumors reached 80-220 mm^3, mice were dosed i.v. weekly with test articles (orange arrows). Irrelevant TCE is HAxCD3. PDX IHC (Immunohistochemistry) staining for MSLN expression is depicted in top left of each graph.

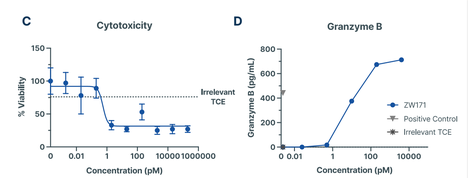
ZW171 Inhibits Growth of Tumor Cells in *ex vivo* Ovarian Cancer Model Leveraging Endogenous Tumor T Cells



B

Tumor sample	Stage IIIB serous adenocarcinoma
Platinum sensitivity	Resistant
MSLN (% of live)	18.9 %
CD3 (% of live)	47.8 %
EpCAM (% of live)	28.9 %
Estimated E:T ratio*	1.7:1

*E:T= % CD3/% EpCAM



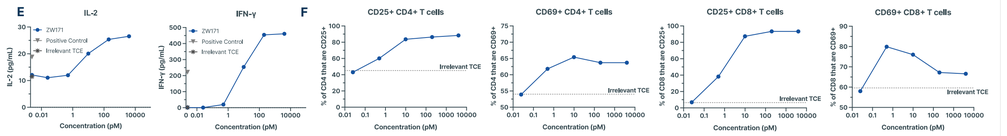


Figure 7. ZW171 induces cytotoxicity, T cell activation and cytokine release in a patient-derived ovarian organoid model. (A) Schematic of ex-vivo study workflow. Organoids were harvested and plated to adhere for 24 h, followed by 72 h treatment with test article and controls. **(B)** Tumor sample information based on histology and patient history. Percentage of live cells from dissociated tumors that were MSLN+, CD3+, or EpCAM+ was measured by flow cytometry **(C)** Cytotoxicity was measured using CTG (CellTiter-Glo®). **(D,E)** Supernatants were collected at endpoint and tested using the Luminex® multiplex assay system to measure cytokines IL-2, IFN-γ, and granzyme B. Positive control: CD3/CD28 T cell activator beads. Irrelevant TCE: HAxCD3. **(D)** CD4+ and CD8+ T cell populations were assessed for CD25 and CD69 expression by flow cytometry, as a measure of T cell activation.

Conclusions

ZW171:

- Mediates potent MSLN-dependent anti-tumor activity in various MSLN-expressing cancers, including ovarian, NSCLC, and pancreatic cancer
- Exhibits strong anti-tumor activity in patient derived organoid with endogenous TIL and in PDX *in vivo* NSCLC and pancreatic models
- Maintains activity in presence of shed MSLN
- Demonstrates enhanced anti-tumor activity compared to other MSLN targeting bispecific TCE

ZW171 is being evaluated in a Phase 1 clinical trial in MSLN-expressing solid tumors (NCT06523803).

References

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