ZW171, a T Cell-Engaging, Bispecific Antibody for the Treatment of Mesothelin-Expressing Solid Tumors

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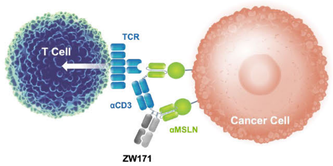
Introduction

Mesothelin (MSLN) is a GPI-linked membrane glycoprotein that is overexpressed in many cancer indications, including pancreatic, mesothelioma, and ovarian[1], for which there is a high unmet medical need. While MSLN-targeting agents have shown early signs of clinical activity, there remains a need for therapies with improved safety and efficacy[2].

T cell engager (TCE) therapies have exhibited clinical utility against hematological malignancies but have shown limited success against solid tumors due to dose-limiting toxicities associated with cytokine release syndrome (CRS) and on-target off-tumor effects[3].

To improve the therapeutic intervention of MSLN-expressing tumors, we utilized the Azymetric™ and EFECT™ platforms and engineering strategies to generate a panel of MSLN-targeting TCEs with a variety of formats, geometries, and paratope affinities. Following extensive screening, a lead candidate with enhanced anti-tumor activity and safety, ZW171, was selected for development.



CD3 Paratope Affinity was Selected to Widen the Therapeutic Window

The ZW-αCD3 paratope binds a discontinuous epitope on CD3ε distinct from the n-terminal epitope of the first- and second-generation versions of the αCD3 paratope broadly used by others, SP34.

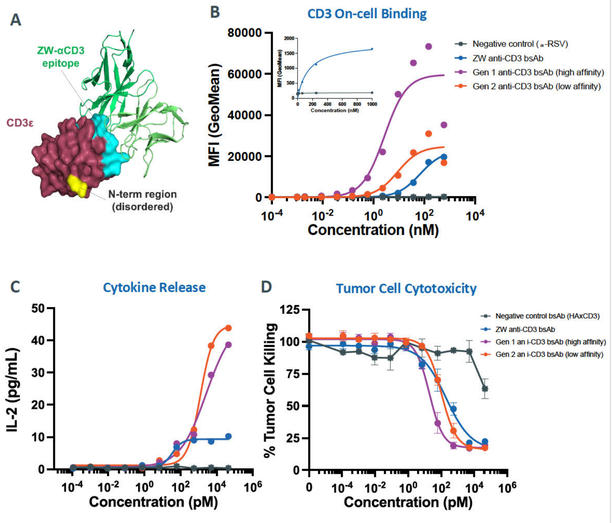


Figure 1. Low affinity ZW anti-CD3 paratope induced potent tumor cell lysis but minimal cytokine release. (**A**) Crystal structure of ZW-αCD3 paratope and CD3 epsilon. (**B**) Binding of bispecific antibodies (bsAb) to CD3 expressed on human panT cells (main figure) and cynomolgus CD8+ T cells (inset) was assessed by flow cytometry. Cytokine release (**C**) and T cell dependent cellular cytotoxicity (**D**) were assessed by co-culturing H292 tumor cells with human panT cells and treating with bispecific antibodies for 3 days. Cell killing was measured using high content imaging with cytokine release measured using a Meso Scale Discovery (MSD) assay.

MSLN Paratope Affinity, Valency, and Geometry of Lead Candidate, ZW171 were Optimized for Enhanced Anti-Tumor Activity

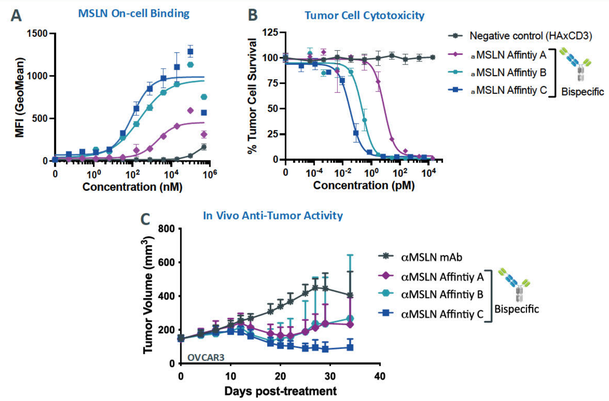


Figure 2. Anti-MSLN paratope affinity was selected to maximize potency. MSLN binding (**A**) was performed on OVCAR3 tumor cells using flow cytometry. T cell dependent cellular cytotoxicity (**B**) was assessed by co-culturing H292 tumor cells with human panT cells and treating with the test articles for 3 days. Cell killing was measured by high content imaging. Anti-tumor activity was assessed in an established OVCAR3 tumor cell in vivo model (**C**). Tumor fragments were engrafted s.c. in NOG mice. Following humanization with PBMCs, mice were dosed with ❋test article b.i.w.x4.

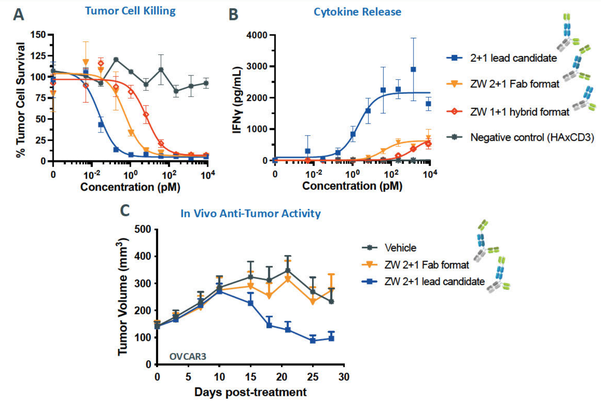


Figure 3. The 2+1 dual scFv bispecific exhibited the most potent in vitro and in vivo anti-tumor activity. T cell dependent cellular cytotoxicity (**A**), cytokine release (**B**) were assessed by co-culturing OVCAR3 tumor cells with human panT cells and treating with the test articles for 3 days. Cell killing was measured by high content imaging, cytokine release by MSD. Anti-tumor activity was assessed in an established OVCAR3 tumor cell in vivo model (**C**). Tumor fragments were engrafted s.c. in NOG mice. Following humanization with PBMCs, mice were dosed b.i.w.x4 with test article.

ZW171 Induces Potent, MSLN-Dependent Cytotoxicity and T cell Activation

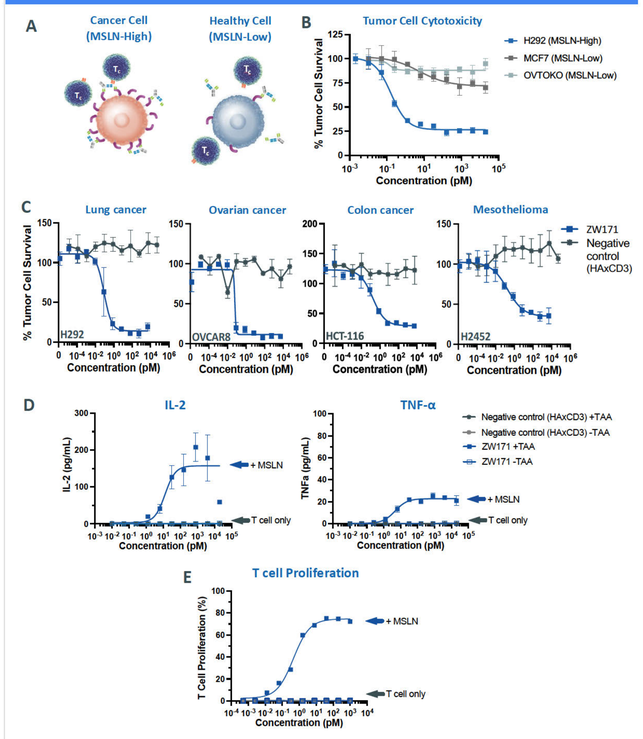


Figure 4. ZW171 induced potent preferential killing of MSLN-overexpressing target cells, and TAA-dependent cytokine release and T cell proliferation. T cell dependent cellular cytotoxicity (**B,C**) and cytokine release (**D**) and were assessed by co-culturing MSLN expressing tumor cell lines with human panT cells and treating with ZW171 or negative control for 3 days. Cell killing was measured by high content imaging, cytokine release was measured by MSD. T cell proliferation (**E**) was assessed by co-culturing CFSE-labeled T cells with OVCAR3 cells and treating with ZW171for 5 days. Proliferation was measured by flow cytometry.

ZW171 Mediates Greater Anti-Tumor Activity Compared to Benchmark in MSLN-Expressing Tumor Models

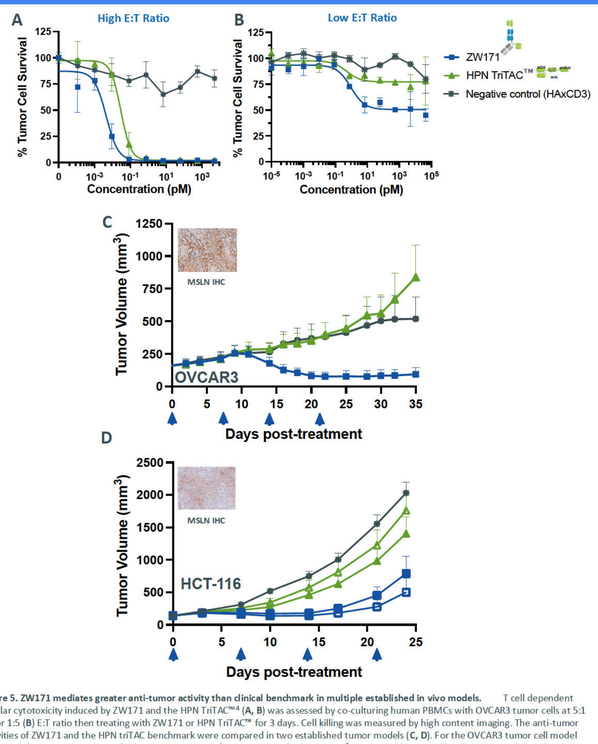


Figure 5. ZW171 mediates greater anti-tumor activity than clinical benchmark in multiple established in vivo models. T cell dependent cellular cytotoxicity induced by ZW171 and the HPN TriTAC™-4 (**A, B**) was assessed by co-culturing human PBMCs with OVCAR3 tumor cells at 5:1 (**A**) or 1:5 (**B**) E:T ratio then treating with ZW171 or HPN TriTAC™ for 3 days. Cell killing was measured by high content imaging. The anti-tumor activities of ZW171 and the HPN triTAC benchmark were compared in two established tumor models (**C, D**). For the OVCAR3 tumor cell model (**C**), tumor fragments were engrafted s.c. in NOG mice. After tumors reached ~100 mm³, mice were humanized with PBMCs and dosed with 3 mg/kg HAxCD3 or ZW171 i.v. QW x4 or i.p. daily x 18 with HPN TriTAC™. For the HCT-116 tumor cell model (**D**) NPG mice were engrafted with HCT116 cells and human PBMC intraperitoneally. After tumors reached ~100 mm³, mice were dosed with 3 mg/kg (■) or 1 mg/kg (□) ZW171 or 3 mg/kg HAxCD3 (❋) i.v. QW x4 or with 0.43 (▲) or 0.14 (△) mg/kg HPN triTAC™ i.p. daily x 18. Serum exposure concentrations and matched exposure doses (3 mg/kg ZW171 vs 0.43 mg/kg HPN TriTAC™; 1 mg/kg ZW171 vs 0.14 mg/kg HPN TriTAC™) confirmed by PK analysis.

ZW171 is Well-Tolerated in Cynomolgus Monkeys

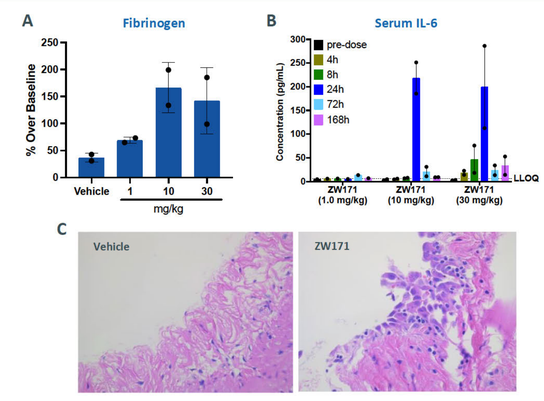


Figure 6. Toxicology findings were mild and associated with the known mechanism of action for ZW171. Cynomolgus monkeys were given a single dose of 1, 10, or 30 mg/kg. Serum levels of fibrinogen (**A**) were elevated compared to baseline in animals treated with ZW171. Transient increases in IL-6 (**B**), MCP-1 and GM-CSF (not shown) were observed. Histopathology was completed to assess microscopic changes to MSLN expressing tissues in response to ZW171 (**C**). Hyperplasia/hypertrophy and inflammation in mesothelium of multiple tissues (stomach shown above) 1 week after dosing with ZW171. Observations were similar at 1, 10, and 30 mg/kg.

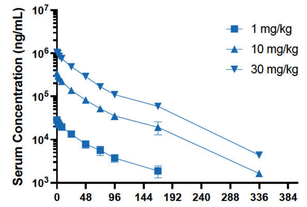


Figure 7. ZW171 Displays Prolonged half-life in NHP. Cynomolgus monkeys were given a single dose of 1, 10, or 30 mg/kg. Following dosing, serum was collected over 14 days and ZW171 concentrations were measured by ELISA.

Conclusions

- ZW171 was selected as a lead candidate through iterative engineering and screening of paratope affinities and formats.
- ZW171 induces potent preferential killing of MSLN-overexpressing target cells and stimulates MSLN-dependent T cell activation, mitigating the risk of on-target-off tumor toxicity and peripheral T cell activation and CRS.
- ZW171 exhibits potent tumor growth inhibition in MSLN expressing tumor models and is well tolerated in cynomolgus monkeys up to 30 mg/kg.
- Collectively, these data suggest that ZW171 could overcome the issues impeding the success of other TCEs developed to treat solid tumors and provide the therapeutic rationale to support the development of ZW171 for the treatment of MLSN-expressing tumors.

References

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