

Design of a Next Generation Tumor Targeted Masked IL-12Fc for Enhanced Tolerability and Localized Anti-Tumor Activity

Nichole K. Escalante; Sifa Arrafi; Andrew Sharon; Scott Lien; Gavin Storoschuk; Ala Ahmed; Mina Khoddami; Alexia Piercey; I-Ting Shao; Anam Lui; Larissa Patlan; Catherine Wu; Richard Kunze; Maya Poffenberger; Chayne Piscitelli; Paul A. Moore; Nina Weissner; Alexey Berezhnoy; Thomas Spreter von Kreudenstein.

Zymeworks Inc, Vancouver, BC, Canada

Abstract
#853

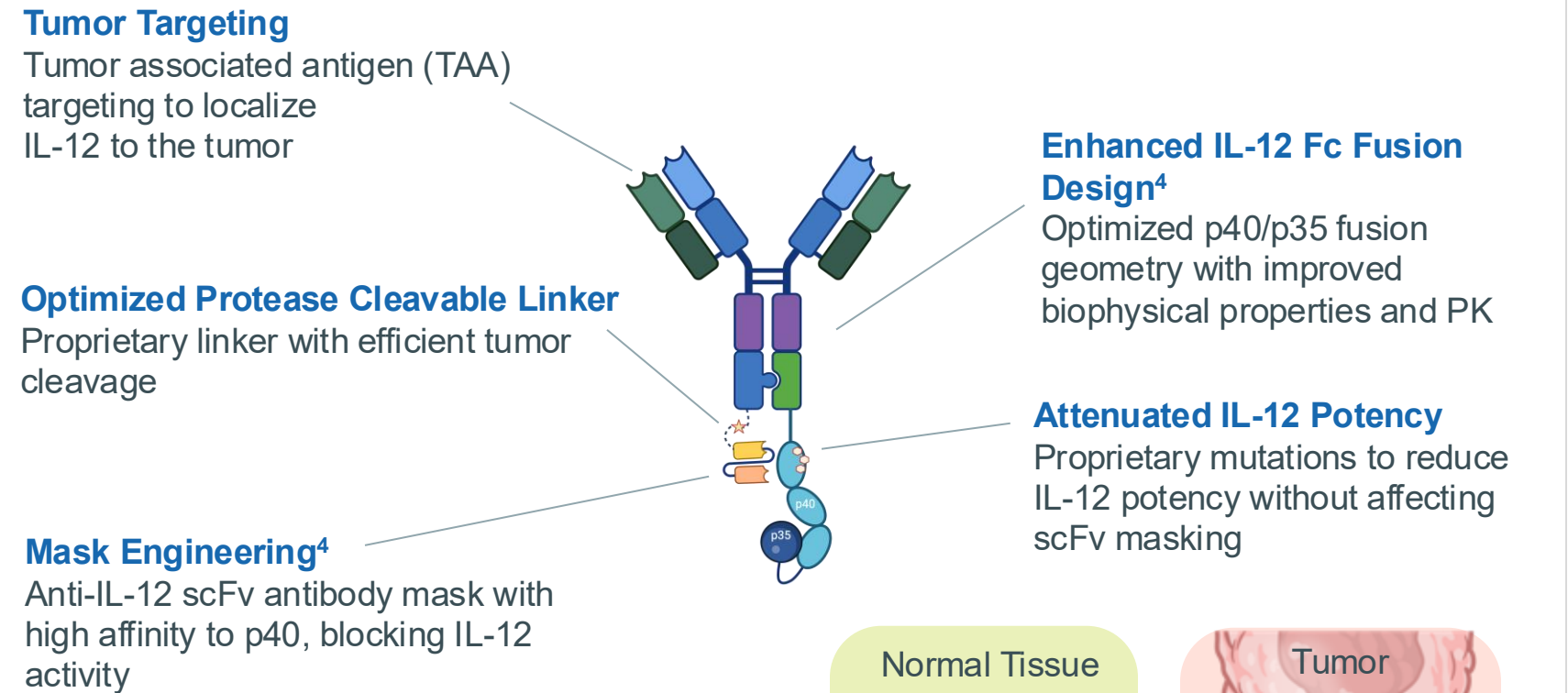


Introduction

IL-12 is a potent immunostimulatory cytokine whose use as an anti-tumor therapeutic has been limited by severe toxicities associated with systemic administration. Tumor targeting and conditional activation in the tumor microenvironment (TME) are two alternative, potentially complementary, strategies to increase therapeutic index of cytokines. To improve the pharmacokinetics and tolerability of IL-12, we have engineered a masked, protease activated human IL-12Fc with attenuated potency (ZW270). ZW270 is engineered with an internally developed, highly efficient cleavage sequence to provide conditional activation of IL-12Fc in a protease-rich tumor environment. Previous data has shown efficient masking of IL-12Fc activity, improved pharmacokinetics and an expanded therapeutic index of ZW270 in vivo compared to wildtype IL-12Fc. ZW270 was well tolerated in non-human primates (>30 mg/kg) with slow extended release in the serum. While this data demonstrates the benefits of the above combined strategies, additional benefit could be gained by targeting ZW270 to the tumor environment, increasing retention time and further localizing IL-12 activity.

To delineate the advantages of targeting a conditionally masked IL-12Fc to the tumor environment, with a tumor targeting Fab, we developed a surrogate masked, protease activated, potency attenuated, mouse IL-12Fc, mZW270. The potency, efficacy and tolerability of a targeted mZW270 was evaluated in vitro and in syngeneic tumor models. Targeted mZW270 demonstrated efficient masking and attenuated potency, similar to ZW270, in in vitro splenocyte and reporter gene assays. In vivo, compared to wildtype mouse IL-12Fc (no masking, attenuation or targeting), it was better tolerated with no early severe bodyweight loss, highlighting the advantage of combined masking and attenuation strategies. In syngeneic and transgenic models, with varying tumor antigen densities, targeted mZW270 activated a local tumor immune response, leading to strong anti-tumor activity, lowering the minimum effective dose without decreasing the tolerability. Initial characterization of a targeted ZW270 indicated translatability of these observations to a human therapeutic. Overall, our combined strategies of masking, potency attenuation and tumor targeting can be incorporated to widen the therapeutic index of an IL-12 based therapeutic.

Next Generation ZW270 Combines Tumor Targeting, Conditional Masking and Attenuation to Widen the Therapeutic Index (TI)



Tumor-targeted ZW270 Binds to TAA-expressing Tumor Cells and Can be Activated by Tumor-associated Proteases

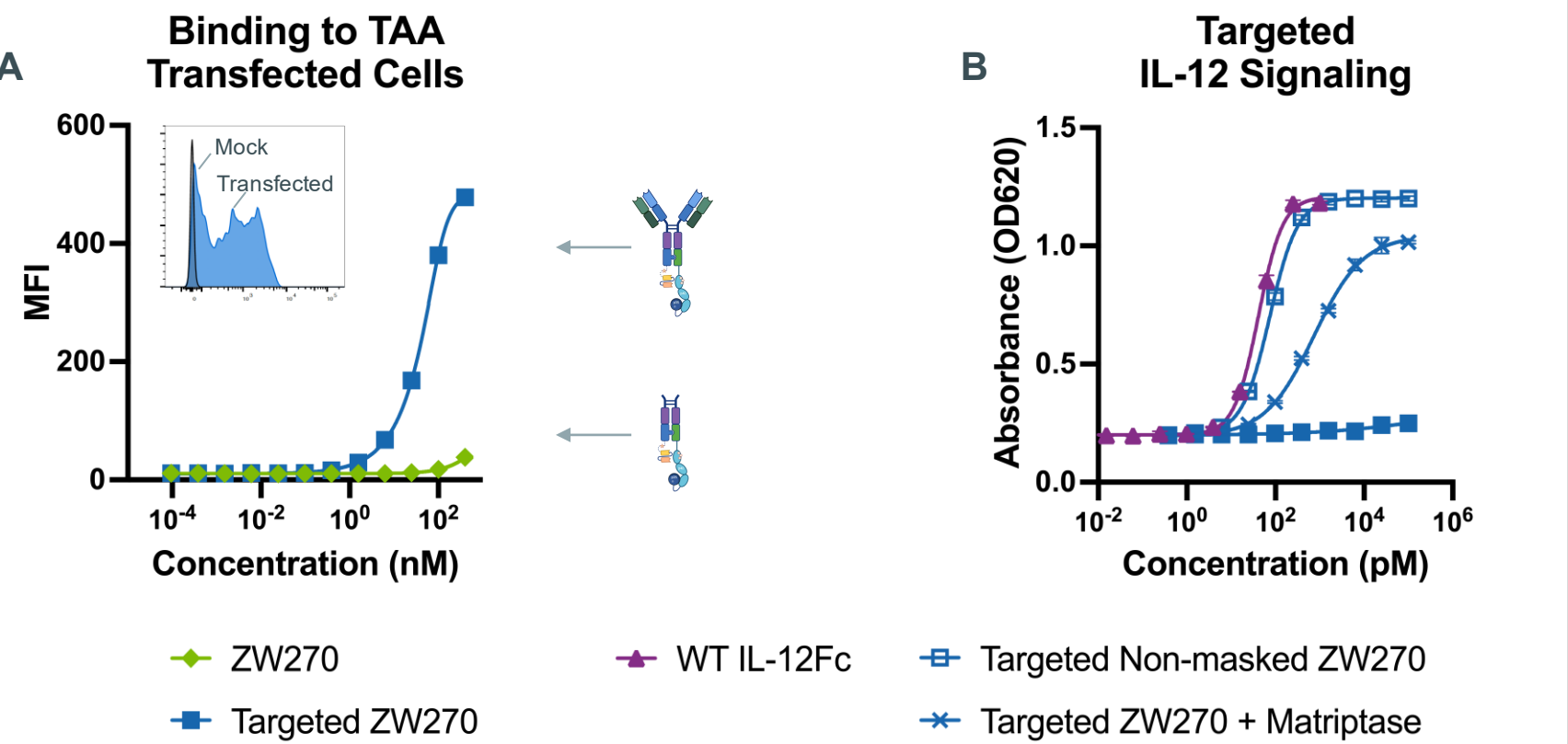


Figure 1. (A) CHO-S cells transfected with human TAA were used to evaluate on-cell binding of targeted and non-targeted ZW270 by flow cytometry. (Insert) Targeted mZW270 binding to mock (grey) and TAA transfected (blue) CHO-S (B) Targeted ZW270 was treated with matriptase and IL-12 activity was measured using a HEK-Blue reporter assay.

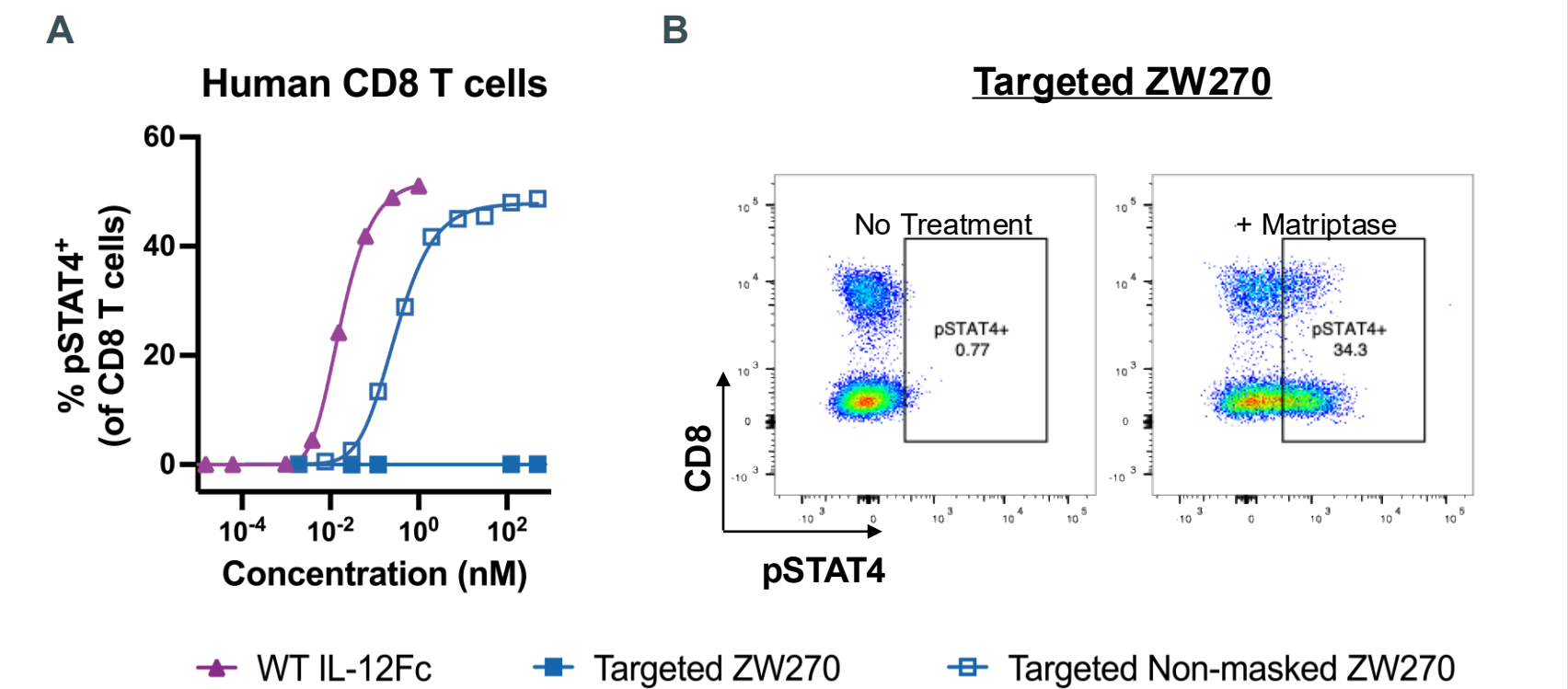


Figure 2. (A & B) Human T cells were activated by CD3/CD28 Dynabeads, treated and activity of targeted ZW270 was measured by pSTAT4 phosphoflow. (B) Targeted ZW270 was treated with matriptase prior to treatment of CD8 T cells.

Conclusions

- Tumor-targeted masked attenuated IL-12Fc (ZW270) binds to TAA-expressing tumor cells and can be activated by tumor-associated proteases
- Mouse homologous agent mZW270 recapitulates properties of masked, protease activated human IL-12Fc with attenuated potency (ZW270)
- Combination of potency attenuation and masking increases tolerability of mZW270; tumor targeting increases anti-tumor activity in vivo

Combined strategies of masking, potency attenuation and tumor targeting of IL-12Fc can be incorporated to widen the therapeutic index of an IL-12 based therapeutic.

Tumor-targeted Mouse mZW270 Induces a Proinflammatory Tumor Response Characteristic for IL-12

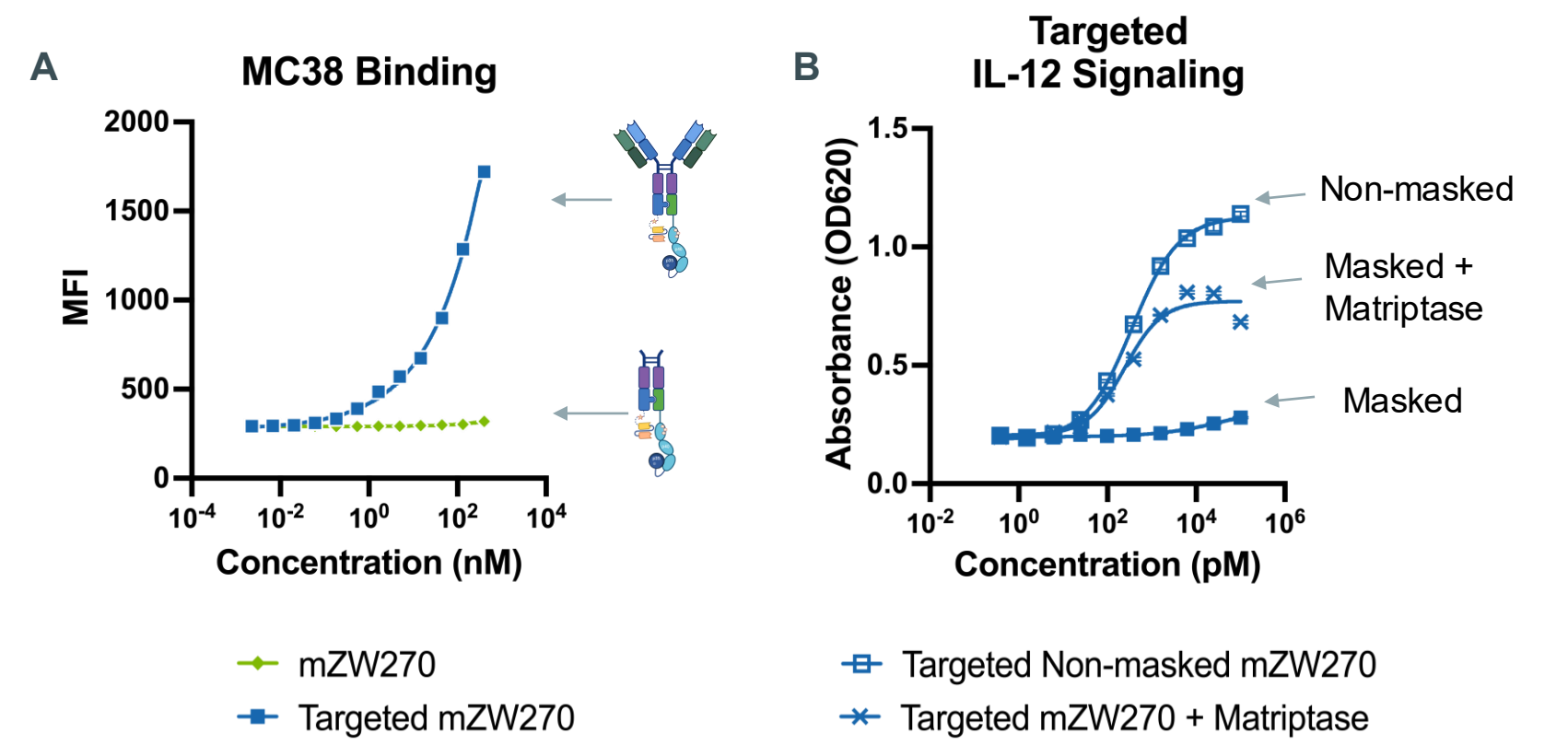


Figure 3. (A) On cell binding on TAA expressing MC38 murine colon adenocarcinoma cells. (B) IL-12 signaling measured by HEK-Blue IL-12 reporter assay after matriptase treatment of targeted masked mZW270.

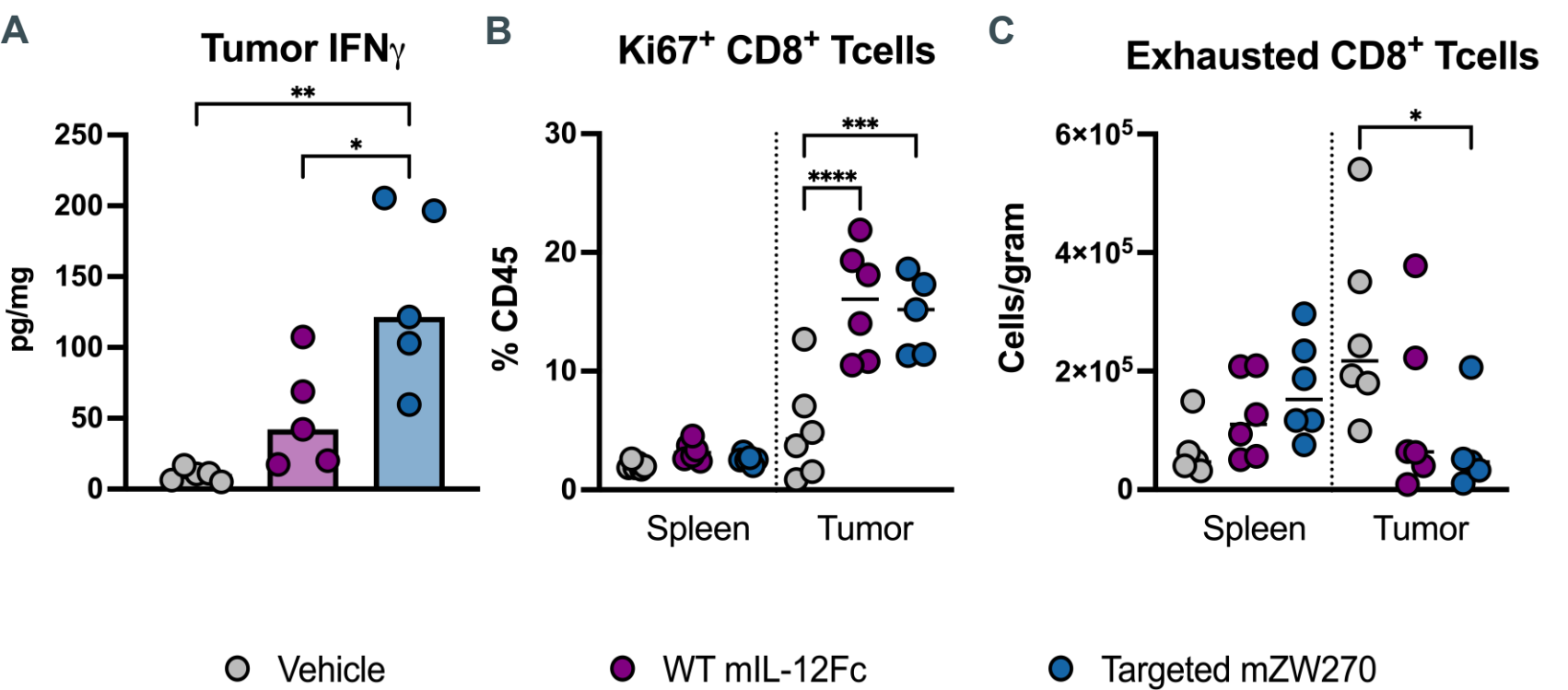


Figure 4. C57Bl/6 mice were inoculated SC with MC38 cells, treated with 4 mg/kg of targeted mZW270 or 5 ug/kg of WT mIL-12 IV and tumors were collected 7 days post treatment. (A) Tumor IFN γ was quantified via MSD. (B, C) Indicated T cell populations in the spleen and tumor were quantified by flow cytometry. Tukey's multiple comparison test * < 0.05, ** < 0.005, *** < 0.0005, **** < 0.00005.

Tumor-targeted mZW270 Has Expanded Therapeutic Index

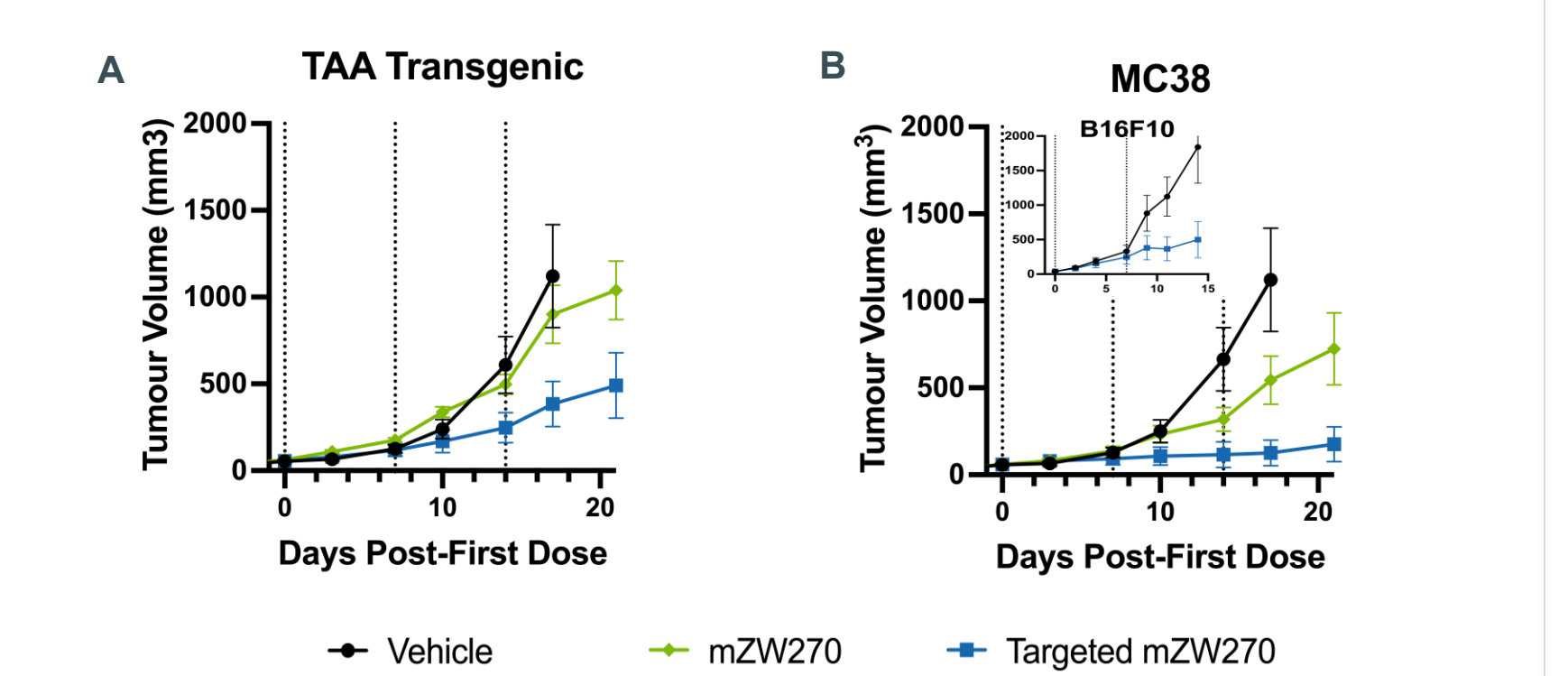
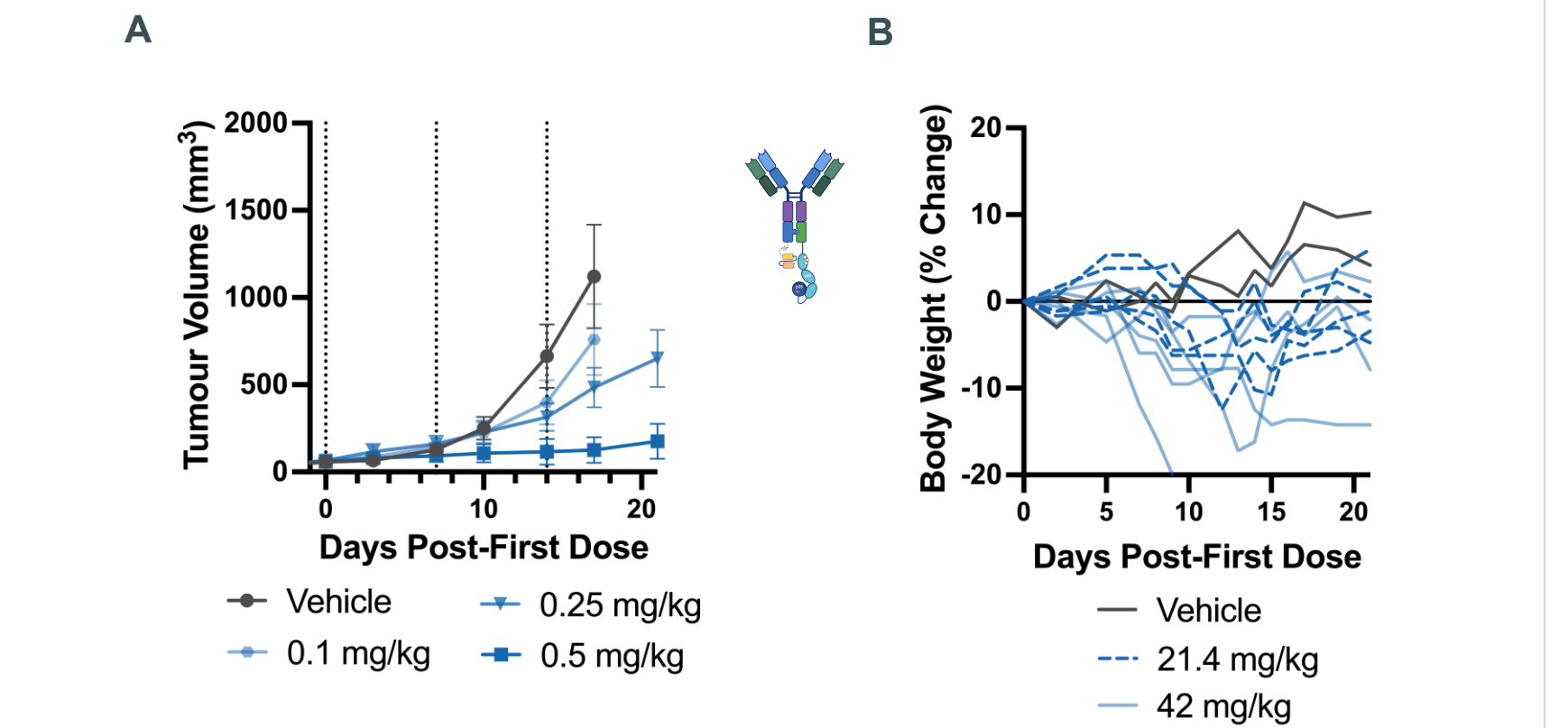


Figure 5. C57Bl/6 mice were inoculated SC with (A) transgenic TAA-expressing (B) MC38 or (insert) B16F10 cells and treated with 0.5 mg/kg (A & B) or 2 mg/kg (insert) IV q1w. Vertical dotted lines indicate treatment days.



Molecule	MTD	MED	TI (MTD/MED)
WT IL-12Fc	0.01 mg/kg	0.005 mg/kg	2
mZW270	16 mg/kg	1 mg/kg	16
Targeted mZW270	21.4 mg/kg	0.5 mg/kg	42.8

Figure 6. (A) C57Bl/6 mice were inoculated SC with MC38 and treated IV q1w to determine minimum effective dose (MED). (B) Tumor-free C57Bl/6 mice were treated IV on days 0 and 7 to determine maximum tolerated dose (MTD).

References

- Elia D. Tait Wonjo et al. Immunity 2019; 50:851-870.
- Khue G. Nguyen et al. Frontiers In Immunology 2020; 11:575597.
- Poffenberger et al. Cancer Res 2023; 83 (7_Supplement): Abstract 2935.
- Jennifer L. Bishop et al. Cancer Res 2021; 81 (13_Suppl): Abstract 1788

Acknowledgements

This study was sponsored by Zymeworks BC Inc.

SITC
2025

