Increasing The Therapeutic Index Of IL-12 By Engineering For Tumor Specific Protease Activation

Abstract: 1788

Jennifer L. Bishop[1]; Ryan Blackler[1]; Gesa Volkers[1]; Maya Poffenberger[1]; Irene Yu[1]; Joel Smith[1]; Akram Khodabandehloo[1]; Sifa Arrafi[1]; Desmond Lau[1]; Liz Stangle[1]; Leisa Stenberg[1]; Patricia Zwierzchowski[1]; Iulia Dude[1]; David Douda[1]; Grant Wickman[1]; Jeff Proctor[1]; Gerry Rowse[1]; Laurence Madera[1]; Genevieve Desjardins[1]; Nicole Afacan[1]; Stuart Barnscher[1]; David Mills; Thomas Spreter[1]; Surjit Dixit[1]. (Corresponding author: jennifer.bishop@zymeworks.com)

1. Zymeworks Inc., Vancouver, BC, Canada

Background

- IL-12 is a pleiotropic cytokine produced by innate immune cells that potently stimulates anti-tumor cytotoxic, T helper and NK cell mediated immunity[1]
- IL-12 significantly reduces tumor growth in multiple syngeneic mouse models, but the efficacy of IL-12 has been limited by toxicity in clinical trials[1,2]
- Protease dependent activation of therapeutics with high on-target, off-tumor toxicities may be used to localize activity to the tumor microenvironment, which is enriched with specific proteases compared to normal tissues[2,3]

- We propose to widen the therapeutic index of this highly potent cytokine by engineering blocked IL-12 fusions designed to be activated by proteases in the tumor microenvironment while maintaining low cytokine activity in peripheral tissues, resulting in a well-tolerated anti-cancer immune response

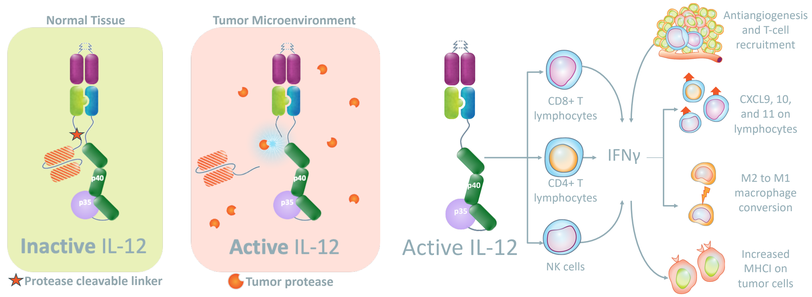


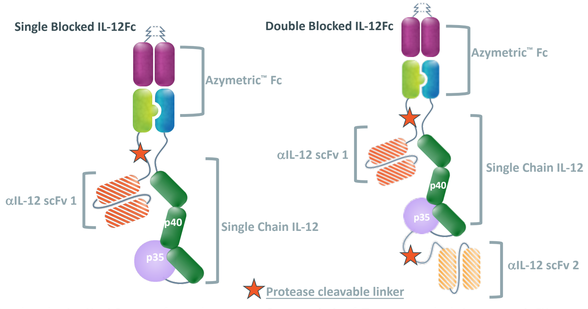
Figure 1: Proposed mechanism of protease dependent activation of Zymeworks' IL-12Fc fusions specifically within the tumor microenvironment and generation IL-12 dependent anti-tumor immunity[4]

Engineering Antibody Blocked IL-12 Fc For Protease Dependent, Tumor Specific Activation

- To improve both tolerability and efficacy of IL-12, we engineered IL-12Fc fusions with anti-IL-12 antibodies that block IL-12 activity
- Using linkers designed to be cleaved by highly active intratumoral proteases, blocking antibodies are released specifically in the tumor microenvironment, thereby increasing intratumoral IL-12 activity

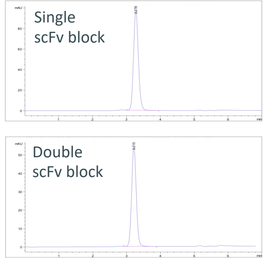
Figure 2: Antibody blocked IL-12Fc characterization.

A



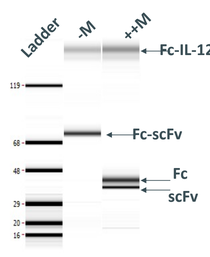
A. Single chain IL-12 was fused to one C-termini of Zymeworks' Azymetric™ Fc heterodimer. To the other Fc C-termini or from p35, an anti-IL-12 scFv was fused via a protease-cleavable linker in order to block IL-12 activity.

B



B. UPLC-SEC shows single or double ScFv antibody blocked IL-12 purification yields high amount and purity.

C



C. CE-SDS shows single antibody blocked IL-12Fc fusions are cleaved in vitro by recombinant human matriptase.

Protease Cleavable Antibody Blocks- Reduce IL-12Fc *In Vitro* Potency by Up To 100,000-Fold

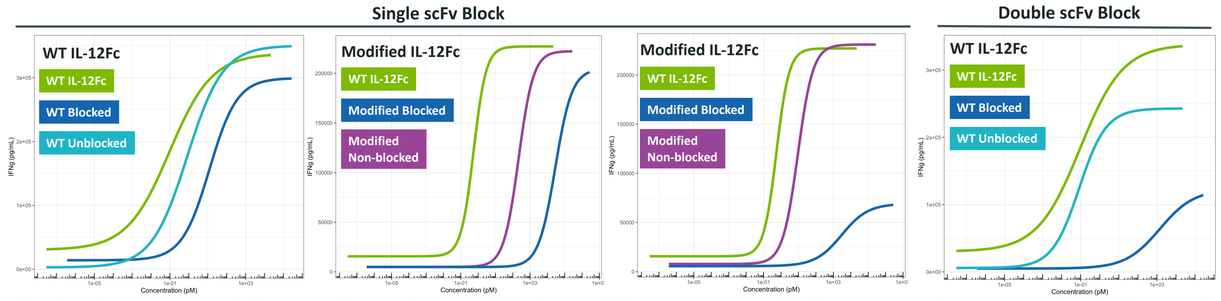


Figure 3 : Antibody blockade reduces IL-12Fc dependent IFNγ production by human CD8T cells. Human CD8T cells were stimulated with CD3/28 beads and treated with varying concentrations of IL-12Fc fusions for 24 hours and IFNγ production was assessed from cell supernatants by ELISA. Blocked wildtype (WT) or modified IL-12Fc fusions contain a single or two (double) ScFv blocking modules. ScFv blocks were removed in unblocked IL-12Fc fusions by protease treatment prior to addition to CD8T cells. Non-blocked and IL-12Fc fusions do not contain any blocking module.

- Single-chain IL-12Fc is a highly potent stimulator of IFNγ production by human CD8T cells
- Single or double scFv blocks reduce potency of IL-12Fc on CD8 T cell IFNγ release between **130-100,000-fold**
- WT IL-12Fc potency can be restored by protease dependent activation by the removal of antibody blocks
- Modifications to non-blocked IL-12Fc alone reduce potency up to 500-fold and can synergize with antibody blocks

Human Tumor Associated Proteases Cleave Antibody Blocks from IL-12Fc Fusions

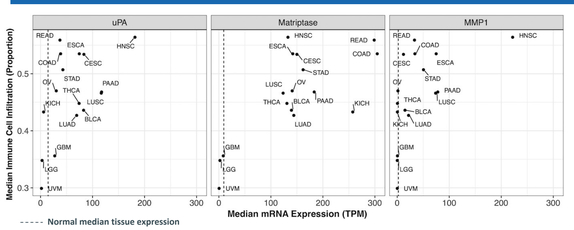


Figure 4: Solid Human Tumors Co-Express High Levels of Proteases And Are Infiltrated By Immune Cells. TCGA RNA sequencing data was analyzed for median expression of protease genes and immune genes indicative of immune cell infiltration.

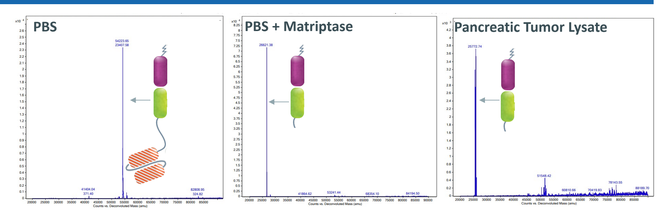


Figure 5: Antibody Blocks Are Removed From Il-12fc Fusions In Human Pancreatic Tumor Tissue Lysate. Single antibody blocked IL-12Fc fusions were incubated in lysates generated from human pancreatic tumor tissue and single Fc + scFv block or Fc alone present in samples were detected by LC/MS.

Conclusions

- Therapeutic index of IL-12 may be fine tuned by the combination of antibody blockade and cytokine modifications that synergize to localize activity to the tumor
- IL-12Fc activity may be localized to the tumor microenvironment via the removal of protease cleavable antibody blocks by tumor specific proteases
- We are pursuing tumor-specific IL-12 fusions for a variety of clinical applications

References

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Acknowledgements

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