ZW270, A Conditionally Masked IL-12 Cytokine Fusion Protein Displaying Potent Anti-tumour Activity Absent of Systemic Toxicity

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Introduction

IL-12 is a pleiotropic cytokine produced by innate immune cells that potently stimulates anti-tumor cytotoxic T and NK cell mediated immunity [1]. IL-12 significantly reduces tumor growth in multiple mouse models, but the efficacy 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 micro-environment but achieving sufficient exposure of activated therapeutic in the tumor micro-environment remains a challenge [2,3]. To widen the therapeutic index of this highly active cytokine, we engineered an attenuated IL-12 that is activated via 'extended release' protease cleavage.

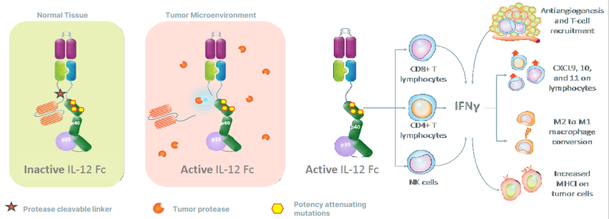
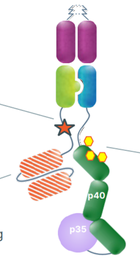


Figure 1: Proposed mechanism of protease dependent activation of Zymeworks' IL-12 Fc in the tumor microenvironment and generation of IL-12 dependent anti-tumor immunity [4].

ZW270 – a masked, 'extended release' protease activated IL-12 Fc with attenuated IL-12 potency

'Extended release' protease cleavable linker
Proprietary protease cleavage linker with efficient tumor cleavage and slow peripheral release (<1%/day)

Mask Engineering[5]
Anti-IL-12 scFv antibody mask with high affinity to p40, blocking IL-12 activity



Optimized IL-12 Fc fusion design[5]
Optimized p40/p35 fusion geometry with improved biophysical properties and PK

Attenuated IL-12 potency
Proprietary mutations to reduce IL-12 potency without affecting scFv masking

Combining Antibody Masking and IL-12 Potency Attenuation Yields Superior Masking Window

- IL-12 was engineered for reduced IL-12Rβ1 affinity and IL-12 potency without impacting binding of the scFv mask.
- In human primary CD8 T cell in vitro assay, ZW270 shows >5,000x reduced potency and superior masking to wild type (WT) IL-12 Fc comparator.

***In vitro* potency of masked and non-masked IL-12 Fc fusion proteins in primary CD8 T cells assay**

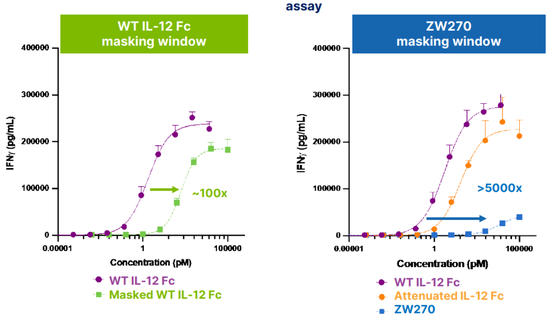


Figure 2: ***In vitro*** **activity of masked vs. non-masked WT IL-12 Fc and ZW270 was evaluated in a human CD8 T cell assay.** Human CD8 T cells were stimulated with anti-CD3/CD28 beads and treated with varying concentrations of IL-12 Fc fusions. IFNg production was assessed from supernatants by MSD assay.

ZW270 Reduces Tumor Growth In Humanized Mouse Model And Is Superior To IL-12 Fc Comparators

- ZW270 and all IL-12 Fc variants were dosed to maximum tolerated dose.

	WT IL-12 Fc	Attenuated IL-12 Fc	Masked WT IL-12 Fc	ZW270
Tumor growth inhibition at highest tolerated dose	✗	✗	✗	✓
Highest tolerated dose (defined by >20% loss of mice)	< 0.0008 mg/kg	< 0.008 mg/kg	> 0.01 mg/kg	> 0.1 mg/kg

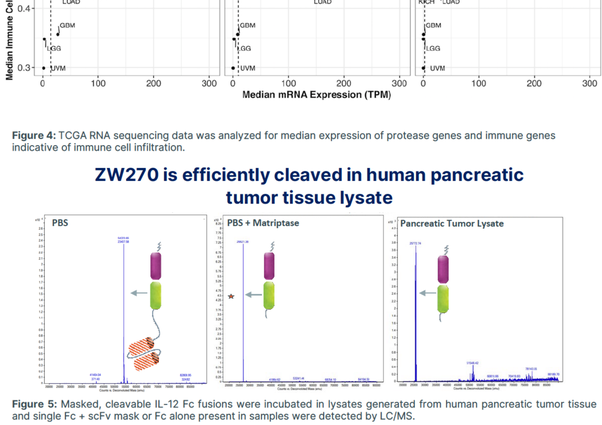


Figure 3: ***In vivo*** **efficacy and tolerability in a human PBMC engrafted xenograft model of human pancreatic adenocarcinoma (BxPC3).** NSG-MHC$^{-/-}$DKO mice were injected with BxPC3 cells SC, followed by IV engraftment of human PBMCs; treatment commenced IV QW x 4 when tumors reached 150-200mm³. Treatment groups and timepoints with >20% loss of mice due to body weight loss after dosing are not plotted.

Human Tumor Associated Proteases Cleave ZW270

Human tumors display high levels of proteases and immune cell infiltration

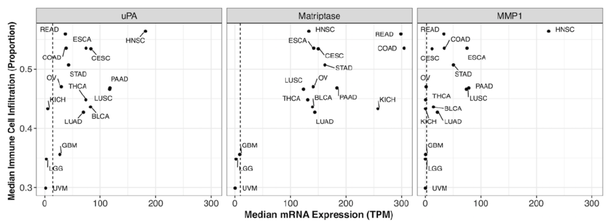


Figure 4: TCGA RNA sequencing data was analyzed for median expression of protease genes and immune genes indicative of immune cell infiltration.

ZW270 is efficiently cleaved in human pancreatic tumor tissue lysate

Figure 5: Masked, cleavable IL-12 Fc fusions were incubated in lysates generated from human pancreatic tumor tissue and single Fc + scFv mask or Fc alone present in samples were detected by LC/MS.

ZW270 Is Well Tolerated in Cynomolgus Monkeys at >10 mg/kg

Single doses of ZW270 at 10 mg/kg and 31.8 mg/kg were well tolerated in cynomolgus monkeys

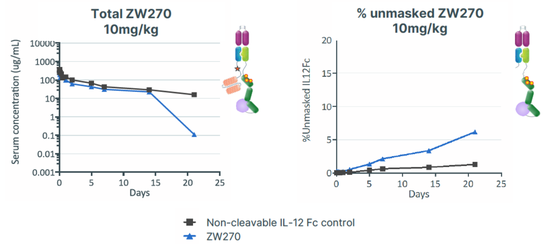
- No mortality or adverse clinical signs were observed at either 10 mg/kg or 31.8 mg/kg.

	WT IL-12 Fc 0.2 mg/kg	ZW270 10 or 31.8 mg/kg
Mortality	Yes at 0.2 mg/kg, day 21	No (up to 31.8 mg/kg)
Clinical signs	Watery feces on Day 15; decreased activity on day 8 and 15; thin day 8 and 15; loose non elastic skin day 15	At 10 mg/kg- no notable changes At 31.8 mg/kg loose feces on day 15
Food consumption Day 3 to 8 (Scale: good-fair-poor)	Fair 3 days; Poor 3 days	Fair 2; Poor 4
Body weight, % difference on day 22 compared to pre-dose	-39.26 %	-7.56 – 13.11%
MTD	0.2 mg/kg	> 31.8 mg/kg

Table 1: In life observations in single dose non-human primate study. IV dose levels from 0.02 mg/kg to 0.2 mg/kg for WT IL-12 Fc and from 0.2 mg/kg to 31.8 mg/kg for ZW270 were tested. WT IL-12 Fc has identical p40/35 fusion geometry to ZW270, but no scFv mask attached.

ZW270 Demonstrates Low Overall Serum Unmasking in NHP and a Gradual 'Extended Release' Mechanism

ZW270 demonstrates low overall unmasking in cynomolgus monkeys and a slow 'extended release' gradual protease unmasking of < 1%/day



Unmasked ZW270 serum concentration at >10 mg/kg is below C_{max} of unmasked WT IL-12 at MTD in cynomolgus monkeys

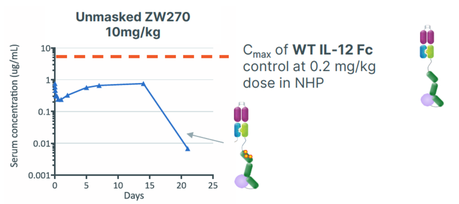


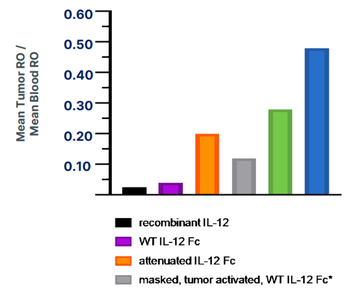
Figure 6: Pharmacokinetics of 10mg/kg single dose ZW270 in cynomolgus monkeys. Serum ZW270 & NC IL-12 Fc were captured with anti-IL-12p35 antibody (total assay) or Briakinumab F(ab') (unmasked assay) and detected with Sulfo-Mouse anti-Human IgG using an MSD based protocol. Non-cleavable IL-12 Fc control has identical fusion geometry to ZW270 with the cleavable sequence replaced by a non-cleavable linker.

QSP Modelling Suggests Superior Projected Therapeutic Index (TI) for ZW270

ZW270 enhanced projected TI is mediated by Fc half-life extension, IL-12 attenuation, and 'extended release' gradual protease unmasking

- ZW270 attenuation allows higher doses & better distribution to tumor.
- ZW270 gradual protease unmasking allows increased distribution to tumor without peak toxicity, further increasing projected TI.

Projected TI Comparison



* masked, tumor activated, WT IL-12 Fc comparator has a peripheral cleavage rate of <0.1%/day, relying on high tumor cleavage for sufficient tumor receptor occupancy

Figure 7: Quantitative Systems Pharmacology (QSP) model was developed combining literature, *in vitro*, *in vivo*, and benchmark data to estimate expected IL-12 receptor occupancies (RO) and human therapeutic index (TI). The toxicity metric is defined as expected *systemic* RO at steady-state; the efficacy metric is expected *tumor* RO at steady-state. All molecules except ZW270 dosed to their projected individual MTD (max systemic RO equivalent to 0.5 ug/kg IV recombinant human IL-12 weekly). ZW270 dosed at 25% MTD.

Conclusions

- ZW270 is a novel, masked 'extended release' protease activated IL-12 Fc fusion with attenuated IL-12 potency.
- ZW270 has potent and superior anti-tumor activity to WT IL-12 Fc and masked WT IL-12 Fc comparators in a humanized mouse model.
- ZW270 is well tolerated in non-human primates to >30 mg/kg single dose.
- Our data suggests that combining two engineering strategies, potency attenuation plus masking with an 'extended release' protease cleavage trigger, has the potential to widen the therapeutic index of IL-12 therapeutics

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