ZW1528, a Bispecific Antibody Targeting IL-4Rα and IL-33, Potently Inhibits Key Mediators of Airway Inflammation

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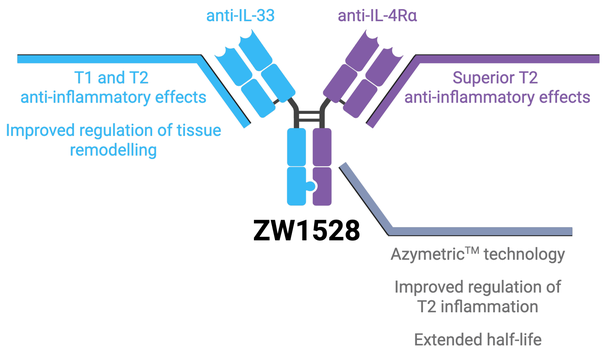
Abstract

Rationale: Chronic obstructive pulmonary disease (COPD) is a difficult-to-treat condition which is characterized by dysregulated type 1/3 (T1/T3) and type 2 (T2) inflammation in the lung. The high prevalence of uncontrolled disease highlights the need to develop therapeutics which can improve outcomes in a larger proportion of patients. IL-33 is a key proinflammatory cytokine associated with airway inflammation and tissue remodelling in COPD. IL-4Rα signalling plays the major role in promoting T2 inflammation and perpetuating disease. ZW1528 is a bispecific antibody targeting IL-4Rα and IL-33 designed for simultaneous blockade of key mediators of airway inflammation.

Methods: ZW1528, an IgG-like bispecific molecule, was constructed using Azymetric™ technology. Binding and blockade of IL-4Rα and IL-33 by ZW1528 was assessed by KinExA™ and reporter gene assays. Primary peripheral blood mononuclear cells (PBMC) were used to determine if inhibition of IL-4Rα and IL-33 signalling translated to regulation of immune pathways linked to airway inflammation. PK studies were performed in rodents and non-human primates (NHP).

Results: ZW1528 binds to IL-33 and IL-4Rα with sub picomolar or low picomolar affinity, respectively, and effectively blocks IL-4Rα and IL-33 signalling at a comparable level to full-sized bivalent monoclonal antibody clinical benchmark controls. The bispecific suppressed IL-4-driven CCL17 production and upregulation of the low affinity IgE receptor CD23 on primary human immune cells in vitro. CD23 blockade was improved compared to bispecific control constructs with Fc-silencing mutations in the IgG1 backbone. In addition, ZW1528 inhibited IFNγ release following IL-33 stimulation of PBMCs. ZW1528 has antibody-like pharmacokinetics (PK) in rodent and NHP model; Fc optimization extended half-life of the molecule. Additionally, our bispecific molecule displays promising biophysical stability at 150 mg/mL to support high concentration dosing and subcutaneous administration.

Conclusion: ZW1528 potently inhibits key mediators of airway inflammation. ZW1528 demonstrated high affinity binding to both IL-4Rα and IL-33 to drive potent blockade of IL-4, IL-13 and IL-33 induced signalling to decrease T2 and T1 immune responses in immune cells. These data highlighting the ability of ZW1528 to block both T1 and T2 inflammation that may translate to better control of COPD in a larger patient population.



ZW1528 has high affinity binding to both IL-33 and IL-4Rα

Target	Human Target Affinity	Cynomolgus Monkey Target Affinity
IL-33	0.23 pM	Yes
IL-4Rα	2.2 pM	Yes, 5.31 pM

Table 1. ZW1528 binds IL-33 and IL-4Rα with high affinity. ZW1528 comprises of high binding affinity arms displaying sub pM and low pM equilibrium dissociation constants (K_D) for human IL-33 and human IL-4Rα, respectively. Binding affinity was determined by KinExA™. *Cynomolgus IL-33 crossreactivity determined by reporter gene assay.

ZW1528 potently blocks IL-4, IL-13 and IL-33 signalling *in vitro*

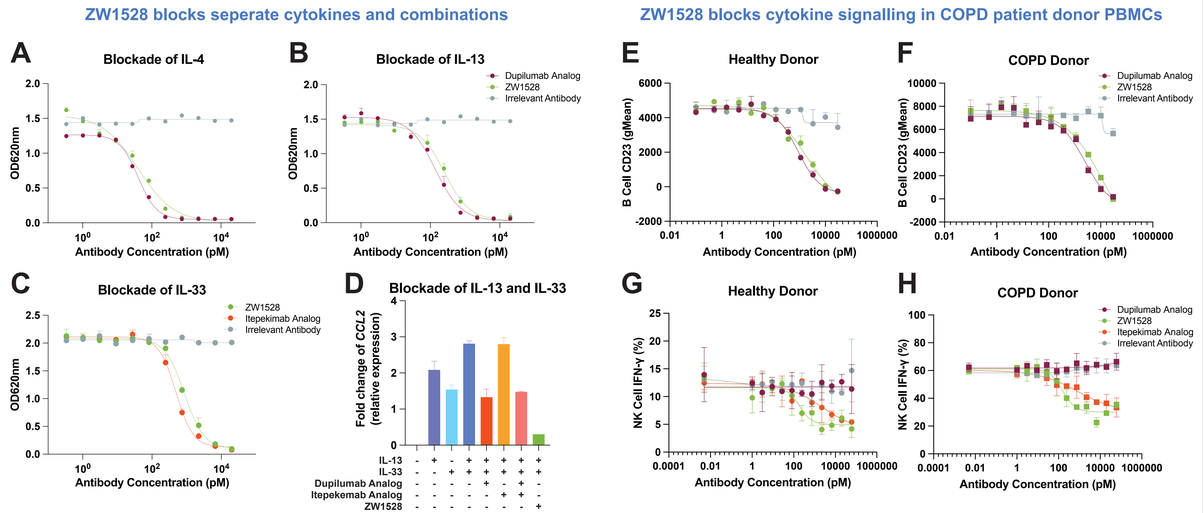


Figure 1. ZW1528 potently blocks IL-4Rα and IL-33 mediated signalling. (A-C) HEKblue cells were incubated with relevant therapeutic anribodies and stimulated with IL-4, IL-13, or IL-33. Reporter gene assays were used to quantify level of cytokine stimulation. **(D)** HEKα cells were incubated with IL-13 and IL-33 along with relevant antibodies for 6 hours. Cytokine stimulation-induced expression of CCL2 was measured by qPCR. ZW1528 outperformed combination therapy of monoclonal benchmarks. **(E/F)** PBMCs from healthy **(E)** or COPD patient **(F)** donors were incubated with relevant antibodies and stimulated with 2ng/mL of IL-4. IL-4Rα stimulation was measured by CD23 upregulation **(G/H)** PBMCs from healthy **(G)** or COPD patient **(H)** donors were incubated with relevant antibodies and stimulated with 10ng/mL of IL-12 and 50nM IL-33. Intracellular staining of IFN-γ was used to measure cytokine stimulation.

ZW1528 reduces sytemic and local lung inflammation in an acute house dust mite asthma mouse model

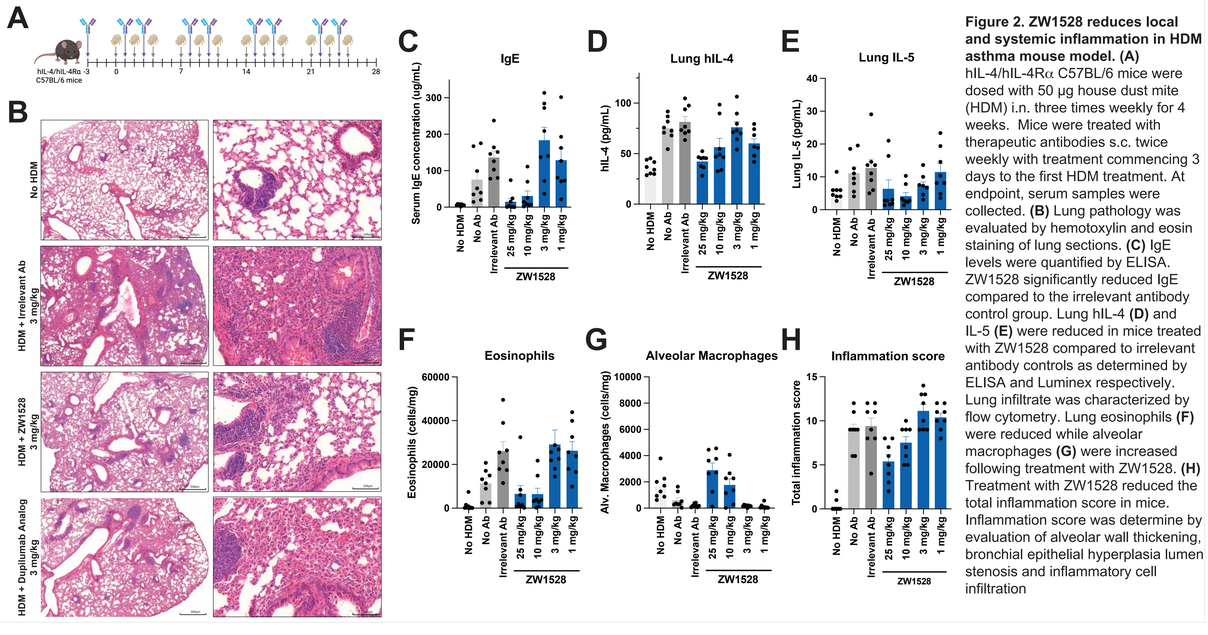


Figure 2. ZW1528 reduces local and systemic inflammation in HDM asthma mouse model. (A) hIL-4/hIL-4Rα C57BL/6 mice were dosed with 50 µg house dust mite (HDM) i.n. three times weekly for 4 weeks. Mice were treated with therapeutic antibodies s.c. twice weekly with treatment commencing 3 days to the first HDM treatment. At endpoint, serum samples were collected. **(B)** Lung pathology was evaluated by hemotoxylin and eosin staining of lung sections. **(C)** IgE levels were quantified by ELISA. ZW1528 significantly reduced IgE compared to the irrelevant antibody control group. Lung hIL-4 **(D)** and IL-5 **(E)** were reduced in mice treated with ZW1528 compared to irrelevant antibody controls as determined by ELISA and Luminex respectively. Lung infiltrate was characterized by flow cytometry. Lung eosinophils **(F)** were reduced while alveolar macrophages **(G)** were increased following treatment with ZW1528. **(H)** Treatment with ZW1528 reduced the total inflammation score in mice. Inflammation score was determine by evaluation of alveolar wall thickening, bronchial epithelial hyperplasia lumen stenosis and inflammatory cell infiltration

ZW1528 reduces lung IL-33 in a chronic HDM model

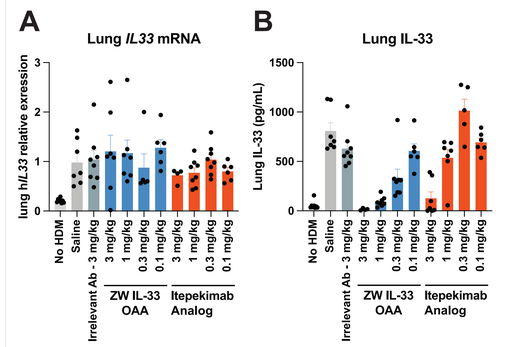


Figure 3. ZW1528 reduces lung IL-33 in chronic HDM mouse model. hIL-33 Balb/c mice were dosed with 50 ug HDM i.n. three times weekly for 15 weeks. Mice were treated with therapeutic antibodies s.c. twice weekly starting at week 4. **(A)** Lung IL-33 gene expression was unaltered with treatment of mice. **(B)** Lung IL-33 protein levels were significantly reduced with treatment of mice.

ZW1528 reduces systemic IgE levels in NHP

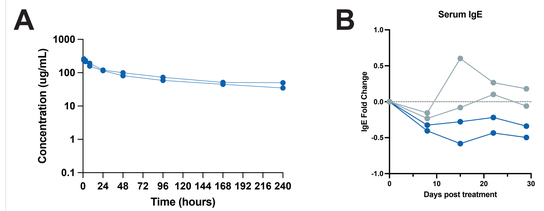


Figure 4. ZW1528 displays antibody like PK NHP and reduces circulating levels of IgE. Cynomolgus monkeys were administered with a single dose of 10mg/kg ZW1528 i.v. Serum was monitored for PK **(A)** and IgE levels over time **(B)**.

ZW1528 has projected extended pharmacokinetics in patients

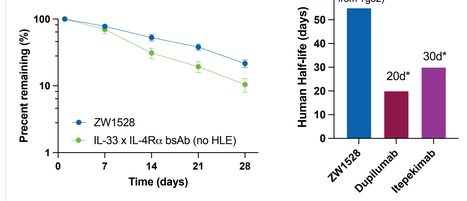


Figure 5. ZW1528 has projected extended pharmacokinetics compared to clincial benchmarks. Tg32 scid mice were injected with 5 mg/kg ZW1528 i.v. **(A)** Serum was monitored for PK. Half-life extending mutations improved PK of ZW1528. **(B)** Allometric scaling was used to predict half life in patients based on Tg32 data.
*based on literature values

Conclusion

ZW1528, an IL-4Rα x IL-33 bispecific antibody displays favourable biology and pharmacology supporting continued development:

- High affinity binding to both IL-4Rα and IL-33 and potent blockade of IL-4, IL-13, and IL-33 *in vitro*.
- Dual IL-4Rα and IL-33 pathway blockade, beyond that achieved by mAb combinations.
- Inhibits Type 2 and non-Type 2 responses *in vitro* in primary immune cells of COPD patients.
- IgG-like PK and biomarkers of target blockade in the NHP.