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

ERS 2025
Abstract number: 4587

Blair K. Hardman, Purva P. Bhojane, Maya C. Poffenberger, Bing C. Wu, Janessa Li, Kesha Patel, Tristan S. Philip, Kurt Stahl, Veronica Luu, Omar Kassas, Steve Booth, Hani Maroki, Shang-Chiung Chen, Nichole K. Escalante, Diana Canals Hernaez, Andrew Sharon, Larissa Patlan Ramirez, Anam Nan Nan Liu, Rachel Guo, Richard Kunze, Gavin Storoschuk, Maya Nathani-Sim, Jenny R. Lu, Paul A. Moore, Thomas Spreter von Kreudenstein, Alexey Berezhnoy

Author Afilliations: Zymeworks Inc., Vancouver, BC, Canada

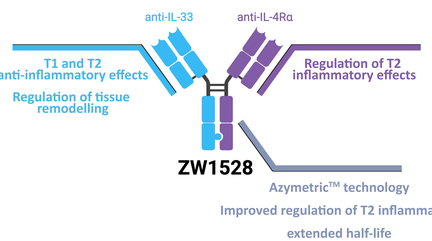
Abstract

Rationale: IL-33 is a key proinflammatory cytokine associated with airway inflammation and tissue remodelling in chronic obstructive pulmonary disease (COPD). In addition, IL-4Rα signalling plays the major role in promoting Type 2 (T2) inflammation in some patients. ZW1528 is a bispecific antibody targeting IL-4Rα and IL-33 designed for dual blockade of key mediators of airway inflammation.

Methods: ZW1528 was constructed using Azymetric™ technology with IgG-like geometry and Fc optimization. Cell cultures and primary immune cells (PBMCs) from healthy donors and COPD patients were used to characterize IL-4Rα or IL-33 blockade in vitro. Pharmacokinetics (PK) and biomarkers of dual blockade were investigated in non-human primates (NHP) and transgenic mice.

Results: ZW1528 has high affinity for IL-4Rα and IL-33 with potent blockade of IL-4/13 and IL-33 signalling comparable to clinical benchmark monoclonal antibody controls. ZW1528 shows in vitro inhibition of T2 and non-T2 responses in PBMCs of COPD patients, illustrated by downregulation of IgE receptors and reduced IFNg release. ZW1528 elicits biomarkers of IL-33 and IL-4Rα engagement in vivo and reduces lung inflammation in murine models. The bispecific has antibody-like PK in rodents and NHPs and extended serum half-life via Fc optimization. Notably, in primary human cell models, ZW1528 shows dual IL-4Rα/IL-33 blockade beyond that seen with clinical benchmark antibody combinations.

Conclusions: These results highlight the ability of ZW1528 to inhibit key cytokines involved in pathology of COPD. This concomitant inhibition may translate into broader suppression of airway inflammation, opening avenues for efficient treatment of patients with COPD.



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

Target	Human Target Affinity	Cynomolgus Monkey Target Affinity
IL-33	0.23 pM	Yes*
IL-4Rα	2.9 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-4Rα and human IL-33, respectively. Binding affinity was determined by KinExA™. *Cynomolgus IL-33 crossreactivity determined by reporter gene assay.

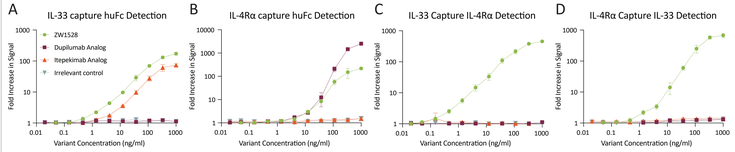


Figure 1. ZW1528 can bind IL-4Rα and IL-33 simultaneously. MSD plates were coated with IL-4Rα or IL-33. ZW1528 (green), Dupilumab analog (purple), Itepekimab analog (orange) and an isotype control (grey) were added to wells. Binding was confirmed by HuFc detection (**A and B**). Other wells had IL-33 (**C**) or IL-4Rα (**D**) added and detected to measure the ability of antibodies already binding antigen coating wells to simultaneously bind the second target.

ZW1528 potently blocks IL-4Rα and IL-33 mediated signalling in vitro

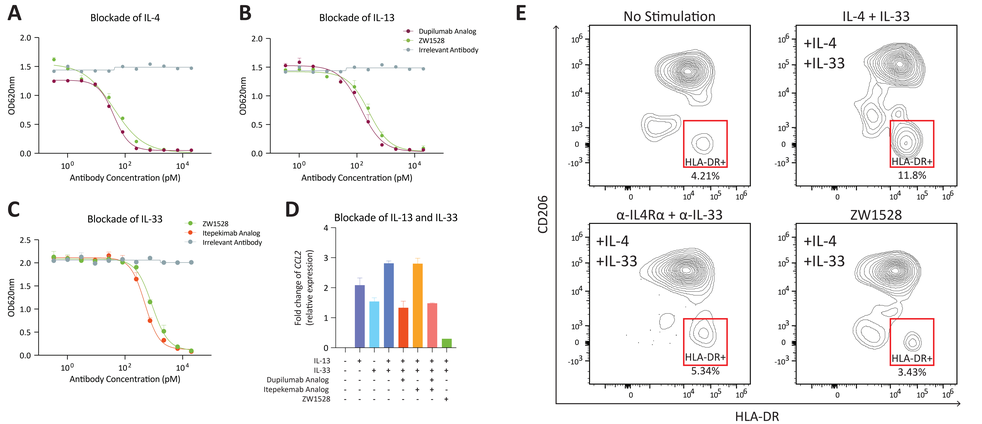


Figure 2. ZW1528 potently blocks IL-4Rα and IL-33 mediated signalling in vitro. (**A-C**) HEKblue cells were incubated with relevant therapeutic antibodies and stimulated with IL-4, IL-13, or IL-33. Reporter gene assays were used to quantify level of cytokine stimulation. (**D**) HEKα cells were incubated with IL-13 and IL-33 along with relevant antibodies for 6 hours. Cytokine stimulation-induced expression of CCL2 was measured by qPCR. (**E**) Monocytes were enriched from PBMCs by adhesion to cell culture dishes. Enriched monocyte cultures were incubated for 72 hours with IL-4, IL-33, and relevant antibodies. Activation of monocytes was characterized via flow cytometry to measure expression of HLA-DR.

ZW1528 reduces inflammation in house dust mite acute asthma mouse model

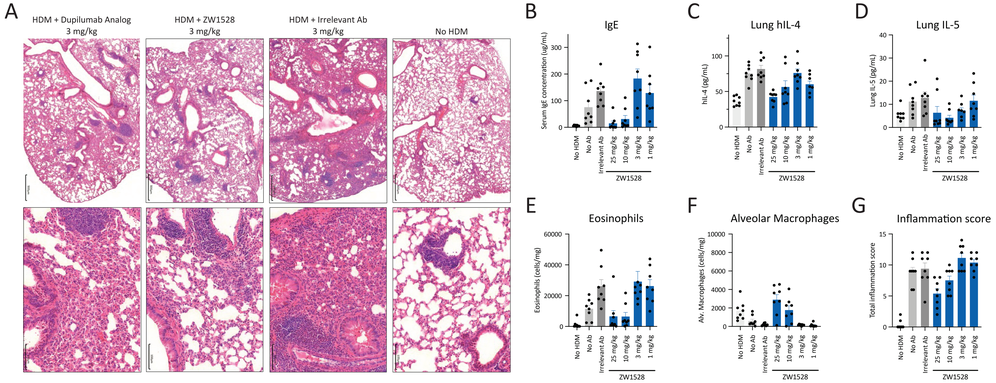


Figure 3. ZW1528 reduces local and systemic inflammation in HDM asthma mouse model. hIL-4/hIL-4Rα C57BL/6 mice were dosed with 50 µg house dust mite (HDM) i.n. three times weekly for 4 weeks. Mice were treated with therapeutic antibodies s.c. twice weekly with treatment commencing 3 days prior to the first HDM treatment. At endpoint, serum samples were collected. (**A**) Lung pathology was evaluated by hemotoxylin and eosin staining of lung sections. (**B**) IgE levels were quantified by ELISA. ZW1528 significantly reduced IgE compared to the irrelevant antibody control group. Lung hIL-4 (**C**) and IL-5 (**D**) 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 (**E**) were reduced while alveolar macrophages (**F**) were increased following treatment with ZW1528. (**G**) 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 blocks cytokine signalling in COPD patient PBMCs

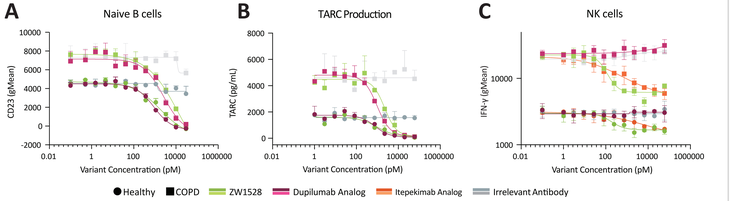


Figure 4. ZW1528 blocks cytokine signalling in COPD patient PBMCs. (**A/B**) PBMCs from healthy or COPD patient donors were incubated with antibodies and stimulated with 2ng/mL of IL-4. IL-4Rα stimulation was measured by CD23 (**A**) and TARC production (**B**). PBMCs from healthy or COPD patient donors were incubated with antibodies and stimulated with 10ng/mL of IL-12 and 50nM IL-33. Intracellular staining of IFN-γ was used to measure cytokine stimulation (**C**).

ZW1528 reduces systemic IgE levels in NHP

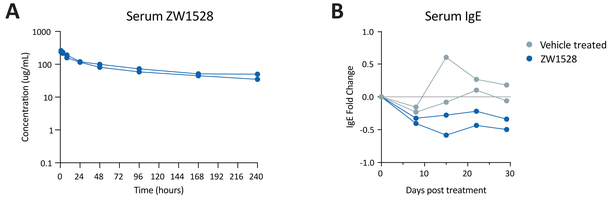


Figure 5. ZW1528 displays antibody like PK NHP and reduces circulating levels of IgE. Cynomolgus monkeys were administered with a single dose of 10mg/kg ZW1528 i.v. Serum was monitored for PK (**A**) and IgE levels over time (**B**).

ZW1528 has projected extended pharmacokinetics in patients

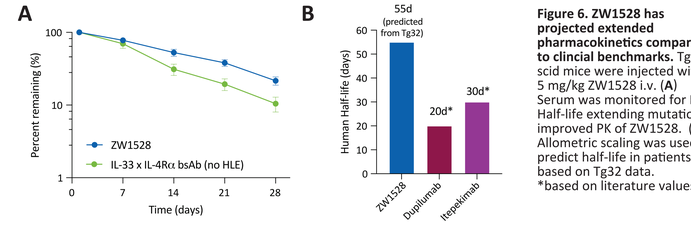


Figure 6. ZW1528 has projected extended pharmacokinetics compared to clincial benchmarks. Tg32 scid mice were injected with 5 mg/kg ZW1528 i.v. (**A**) Serum was monitored for PK. Half-life extending mutations improved PK of ZW1528. (**B**) Allometric scaling was used to predict half-life in patients 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 NHP.

Clinical investigation of ZW1528 on track to initiate in 2026