THE ANTI-IL-33 ANTIBODY, ITEPEKIMAB, POTENTLY BLOCKS AIRWAY INFLAMMATION POST A SINGLE DOSE IN MICE

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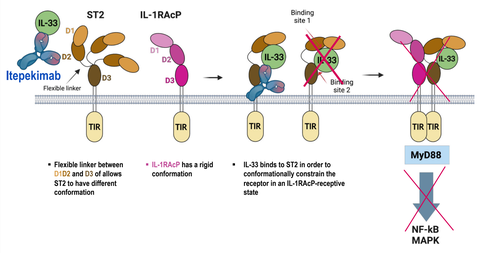
INTRODUCTION

The proinflammatory cytokine, IL-33, signals through a heterodimer of ST2 and IL-1RAcP, and induces MyD88-dependent NF-kB signaling. Blockade of IL-33 using itepekimab, a high affinity fully human IgG4 monoclonal antibody, has shown benefit in early asthma and COPD clinical studies. **Here, we assessed the binding kinetics of itepekimab to human IL-33 and performed pharmacokinetic (PK) and pharmacodynamic (PD) assessment in a house dust mite (HDM)-driven lung inflammation mouse model.**

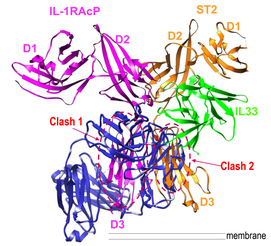
RESULTS

ITEPEKIMAB-BOUND IL-33 CAN BIND TO ST2 BUT PREVENTS TRANSITION TO ACTIVE CONFORMATION REQUIRED FOR RECRUITMENT OF IL-1RACP & SIGNALLING

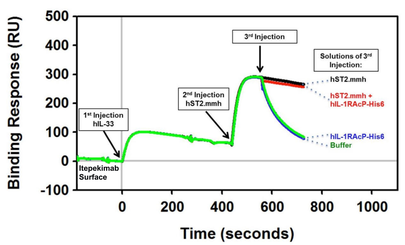
Itepekimab binds to IL33, blocks binding at site 2 and prevents ST2 from forming a rigid conformation that serves as a molecular platform for IL-1RAcP recruitment



Overlay of IL33/ST2/itepekimab Fab cryo-EM structure and IL33/ST2/IL-1RAcP crystal structure (PDB: 5VI4)



Itepekimab complexed with IL-33 can bind ST2



Biacore sequential binding assay demonstrated that IL-33 bound to itepekimab can still bind to ST2 but prevents recruitment of IL-1RAcP

Itepekimab was first captured on an anti-hFc antibody sensor chip, followed by sequential injection of IL-33, soluble ST2 and soluble IL-1RAcP. Itepekimab bound to IL-33 allowed binding to ST2 leading to a ternary complex formation (Itepekimab:IL-33:ST2). No additional binding was observed when IL-1RAcP was injected (blue line) or in the presence of additional ST2 (red line), suggesting that Itepekimab:IL-33:ST2 complex prevents the recruitment of IL-1RAcP

CHRONIC HOUSE DUST MITE (HDM) DRIVEN AIRWAY INFLAMMATION MODEL

HDM or Saline i.n. for 15 weeks

αIL-33 or Control IgG

Il33$^{hu/hu}$ mice

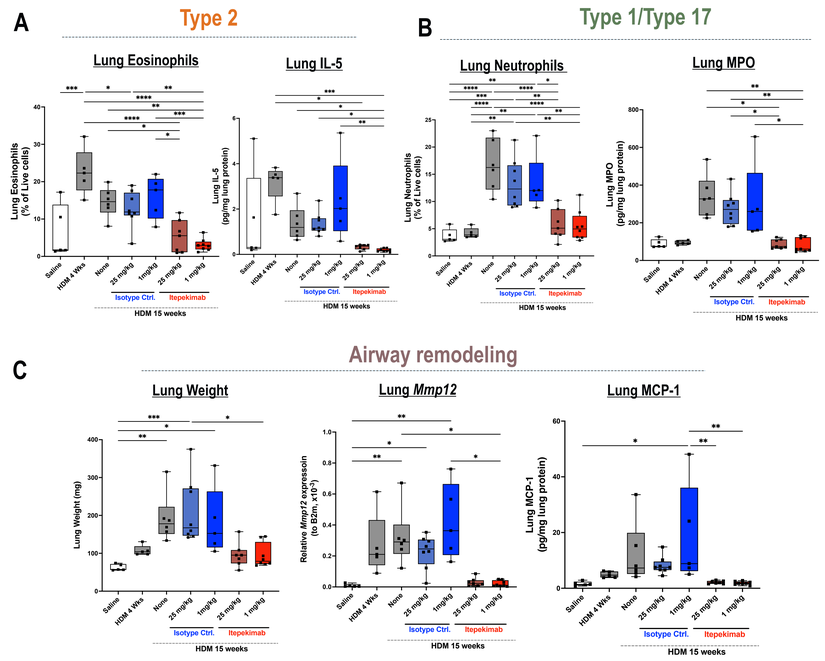
Tissue collection

- Mice (C57BL/6NTac (75%)/129S6SvEvTac (25%)) have genetic replacement of IL-33 with corresponding human sequences
- Intranasal administration of HDM for 15 weeks, 3x/week.
- Therapeutic treatment (25, 1, 0.25, 0.05 mg/kg, 2x/week):
  - **Anti-IL-33 (itepekimab**, huIgG4^{P})
  - Respective isotype controls.

DISCLOSURE

Research sponsored by Sanofi and Regeneron Pharmaceuticals, Inc.
Seblewongel Asrat, Randi Foster, George Scott, Yi Zhou, Vishal Kamat, Ashique Rafique, Dylan Birchard, Li-Hong Ben, Jeanne Allinne, Andrew J. Murphy, Matthew C. Franklin, Pamela Krueger, Matthew A. Sleeman and Jamie M. Orengo are current and former employees of Regeneron Pharmaceuticals, Inc and may hold stock or stock options

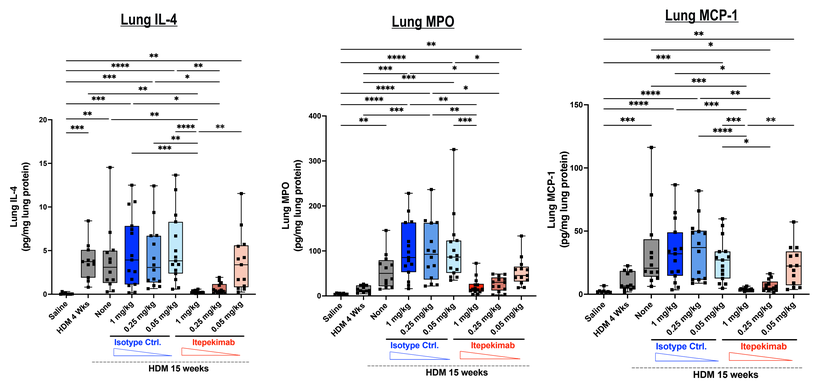
ITEPEKIMAB POTENTLY BLOCKS TYPE 2 AND TYPE 1/TYPE 17 AIRWAY INFLAMMATION AND PREVENTS LUNG REMODELING AT HIGH (25 MG/KG) AND LOW (1 MG/KG) DOSES IN MICE



Itepekimab reduced type 2 and type 1/17 airway inflammation and lung remodeling in mice at high (25mg/kg) and low doses (1mg/kg)

A-C. Il33$^{hu/hu}$ *mice were exposed to HDM for 15 weeks with therapeutic treatment of itepekimab or isotype control at 25mg/kg and 1mg/kg. A. Type 2 airway inflammation was analyzed by looking at lung eosinophilia and lung IL-5 levels. B. Type 1/Type 17 airway inflammation was assessed by looking at lung neutrophilia and lung MPO levels. C. Airway remodeling was assessed by looking at overall lung weight, lung Mmp12 expression and lung MCP-1 levels. Flow cytometry was used to analyze eosinophils and neutrophils. MSD or ELISA was used to analyze lung IL-5, MPO and MCP1 levels. TaqMan was used to measure Lung Mmp12 expression. Each point reflects a single mouse.*

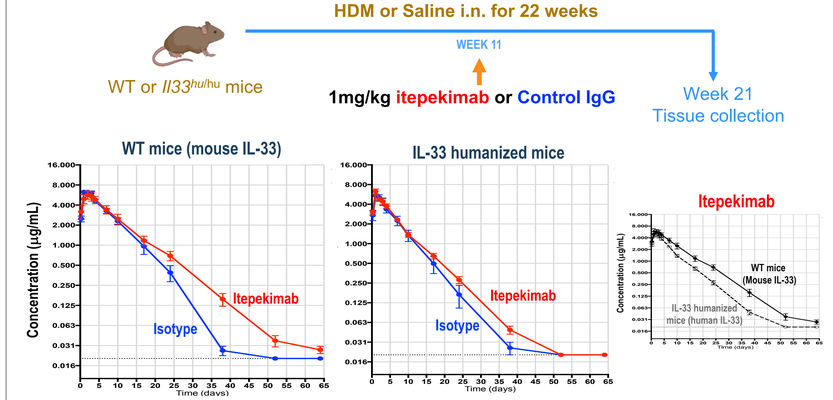
FURTHER DOSE TITRATION HIGHLIGHTS BLOCKING EFFICACY OF ITEPEKIMAB



Itepekimab reduced mixed airway inflammation and remodeling even at low doses (1 and 0.25 mg/kg)

Il33$^{hu/hu}$ *mice were exposed to HDM for 15 weeks with therapeutic treatment of itepekimab or isotype control at 1mg/kg, 0.25mg/kg or 0.05mg/kg. MSD or ELISA was used to analyze lung IL-4, MPO and MCP1 levels. Each point reflects a single mouse.*

A SINGLE THERAPEUTIC DOSE OF ITEPEKIMAB (1MG/KG) SHOWS A COMPARABLE PK PROFILE TO ISOTYPE CONTROL AND SUPPRESSES LUNG CYTOKINES 65 DAYS POST A SINGLE DOSE

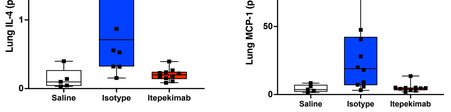


- **Itepekimab has a similar PK profile as IgG4 isotype control in mice humanized for IL-33.**
- **Itepekimab exhibits faster clearance in the presence of target (IL-33 humanized mice) compared to WT mice.**

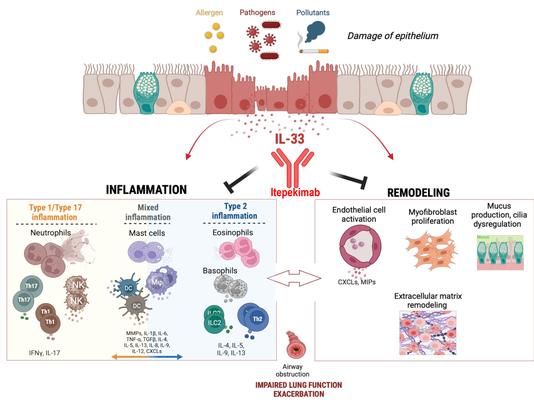
WT or Il33$^{hu/hu}$ *mice were exposed to HDM for 21 weeks and treated with itepekimab or isotype control at 1 mg/kg at week 11. Total circulating mAb levels were measured overtime using human IgG ELISA. Lines represent mean mAb levels (10 mice/group).*

A single dose of itepekimab suppresses lung inflammation 65 days (10 weeks) post mAb administration in a chronic HDM model

Il33$^{hu/hu}$ *mice were exposed to HDM for 21 weeks with 1x therapeutic treatment of itepekimab or isotype control at 1 mg/kg at week 11.*
MSD AND ELISA was used to analyze lung IL-4 and MCP-1 levels at day 65 (10 weeks) post mAb administration. Each point reflects a single mouse.



SUMMARY: IL-33 INITIATES AND AMPLIFIES MIXED AIRWAY INFLAMMATION AND LUNG REMODELING, WHICH IS POTENTLY BLOCKED BY ITEPEKIMAB



IL-33 is a key driver of mixed airway inflammation and remodeling. By its high affinity interaction with IL-33, itepekimab prevents IL-33 from forming an active signaling complex. Intervention with itepekimab, even at doses as low as 1mg/kg, results in reduction of type 1/type 17 and type 2 airway inflammation as well as lung remodeling in a mouse model of chronic progressive lung inflammation