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Rilzabrutinib, a Potent and Selective Bruton's Tyrosine Kinase Inhibitor, Suppresses Reactive Oxygen Species Production and CD11b Activation in Human Eosinophils

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Conflict of interest disclosure

❑ **I have no real or perceived conflicts of interest that relate to this presentation.**

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Rilzabrutinib: A multipronged approach targeting asthma

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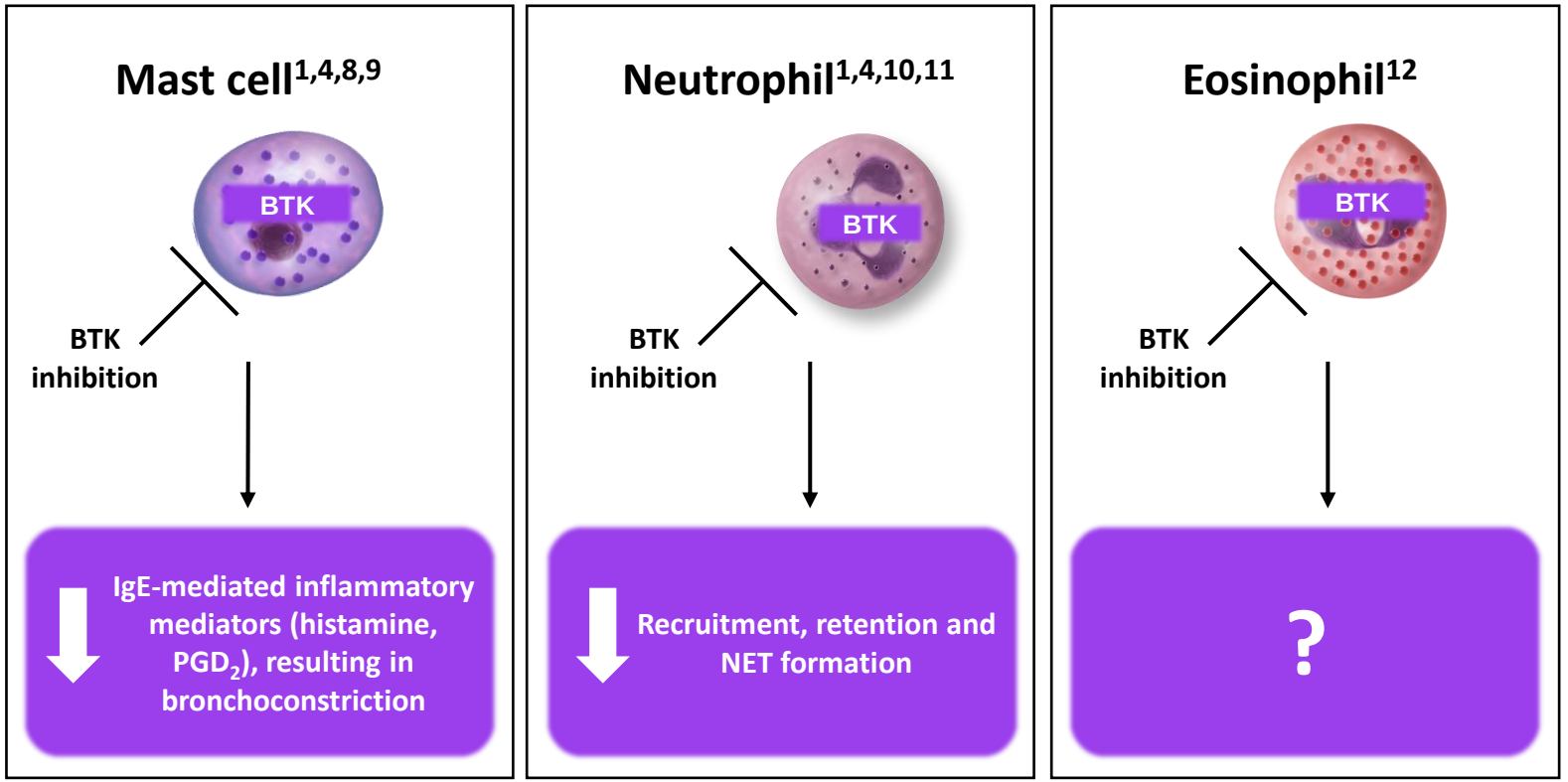
- Bruton's tyrosine kinase (BTK) is an intracellular signaling molecule broadly expressed in many immune cells[1-3]
- BTK-dependent signals play a critical role in multiple asthma disease processes[2-5]
- Eosinophils are key innate immune cells contributing to the pathogenesis of asthma[6,7]
- BTK is expressed in eosinophils; however, the role of BTK in human eosinophils has not been fully investigated[2]



IgE, immunoglobulin E; NET, neutrophil extracellular trap; PGD_2, prostaglandin D2.

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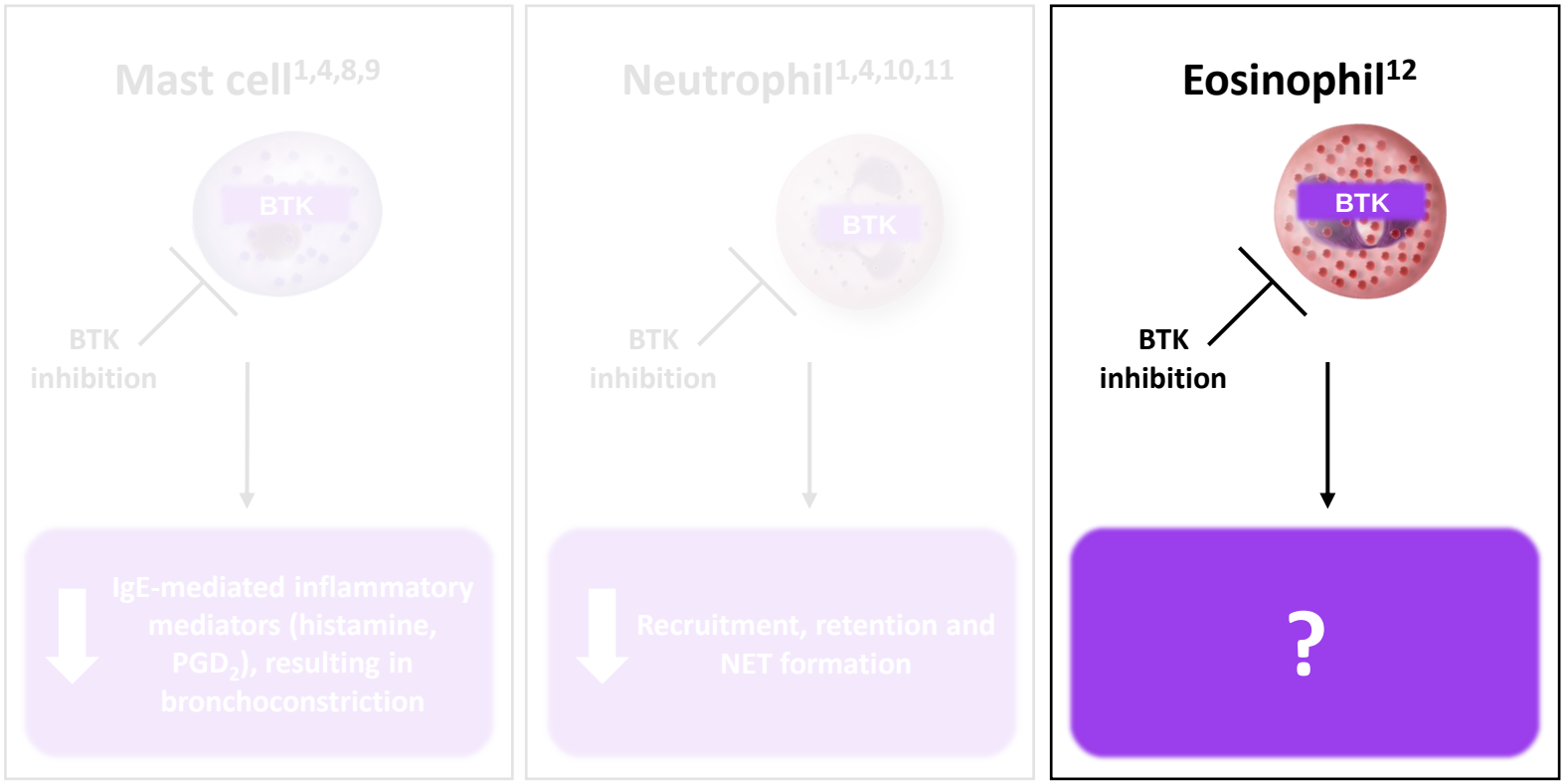


IgE, immunoglobulin E; NET, neutrophil extracellular trap; PGD_2, prostaglandin D2.

Objective

To explore the functional impact of BTK inhibition in human eosinophils using a potent and highly selective inhibitor, rilzabrutinib, which is currently being investigated in clinical trials in asthma

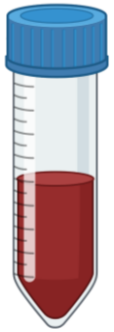
BTK, Bruton's tyrosine kinase.

Rationale: What is the role of BTK in IL-5- and LPS-activated human eosinophils?

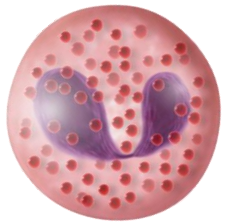
- **IL-5 + LPS** stimulation induced release of eosinophil extracellular traps (EETosis) in eosinophils derived from non-severe and severe eosinophilic asthma patients[13]
- EETosis was associated with **ROS production**[13]
- We explored the impact of BTK inhibition with rilzabrutinib on **IL-5 + LPS-evoked** human eosinophil activation and ROS production

BTK, Bruton's tyrosine kinase; IL-5, Interleukin 5; LPS, lipopolysaccharide; ROS, reactive oxygen species.

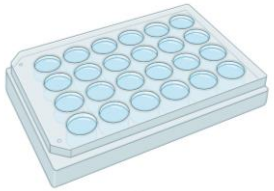
Experimental setup



Whole blood from healthy donors



Eosinophil isolation



Eosinophil activation:

- Pre-treatment with rilzabrutinib (0.01, 0.1, and 1 µM), a BTK inhibitor, for 1 hour before activation
- Activated with IL-5 (20 ng/mL) + LPS (300 ng/mL)

Flow cytometric analysis:

- $CD66b^{+}CD16^{-}$ eosinophils
- ROS indicator (DCF) and CD11b activation

BTK, Bruton's tyrosine kinase; CD, cluster of differentiation; DCF, 2',7'-dichlorodihydrofluorescein diacetate; IL-5, interleukin 5; LPS, lipopolysaccharide; ROS, reactive oxygen species.

Results: Rilzabrutinib significantly inhibited ROS production in eosinophils activated by IL-5 + LPS

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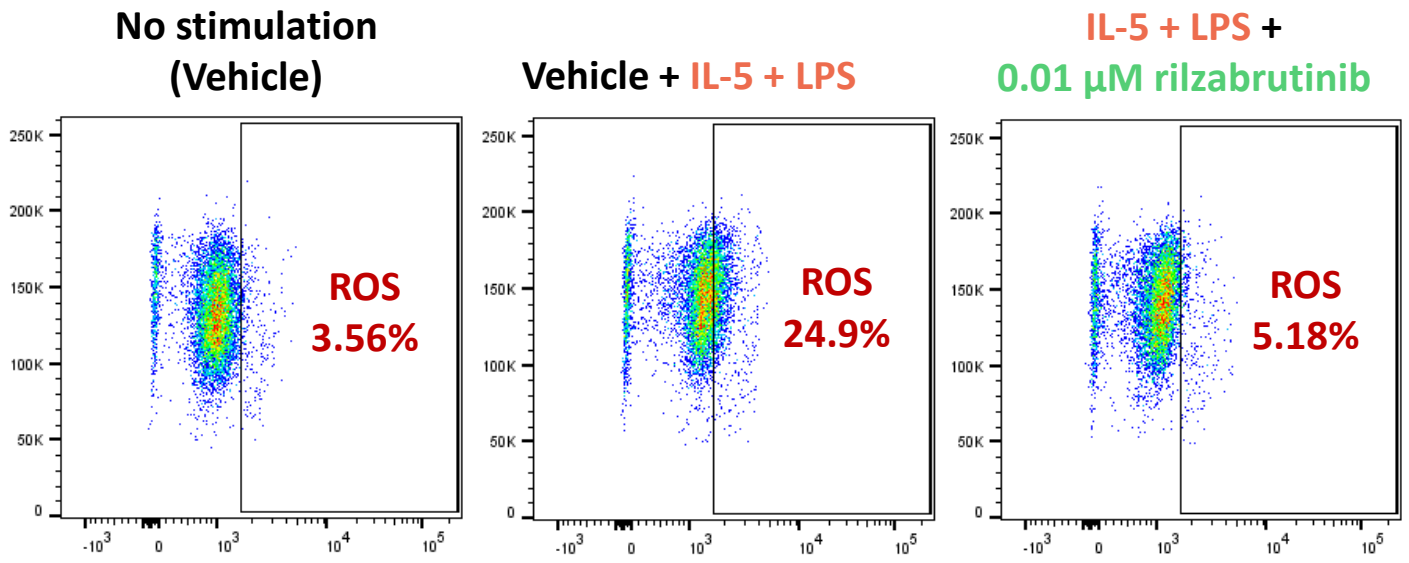
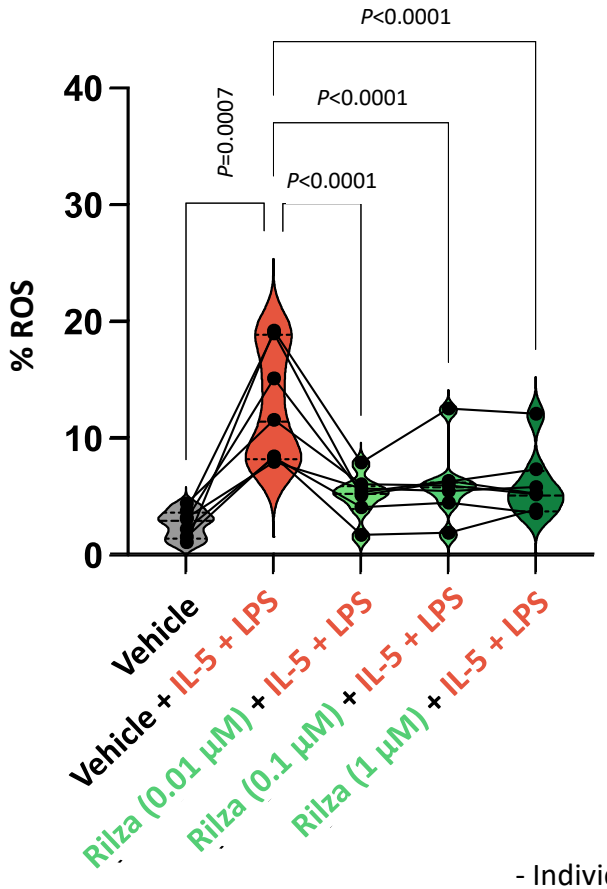
Representative flow cytometry plots



Flow cytometry analysis summary of ROS production



- Individual donor
n=7 donors

IL-5, interleukin 5; LPS, lipopolysaccharide; Rilza, rilzabrutinib; ROS, reactive oxygen species.

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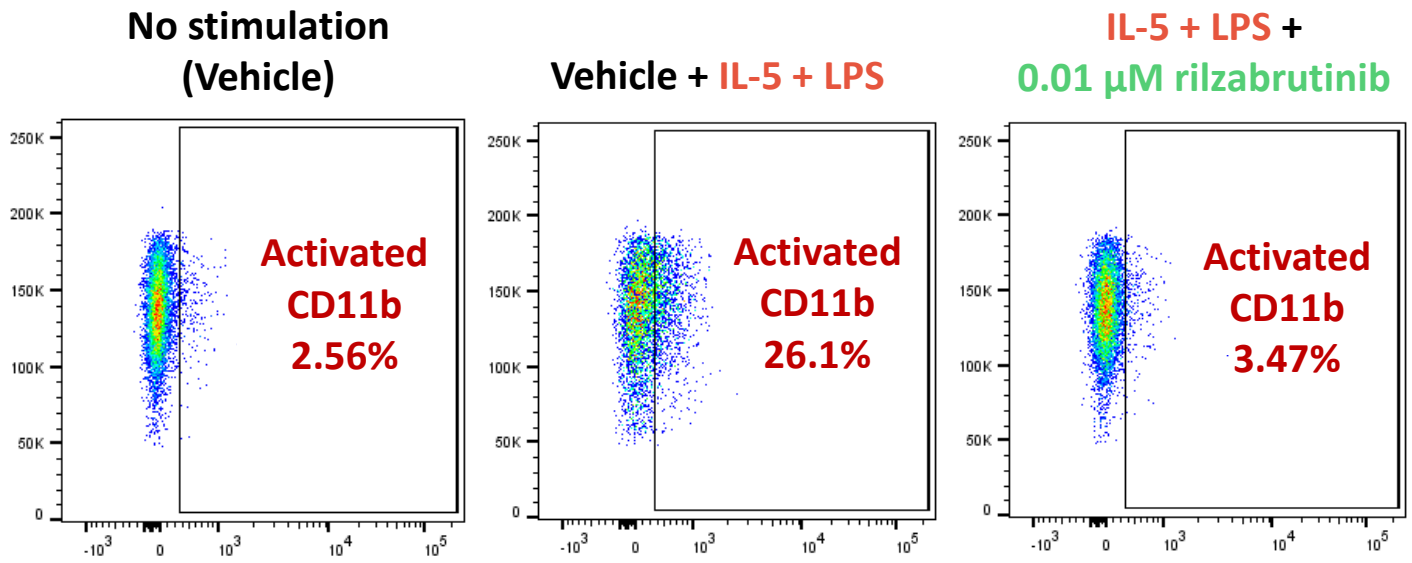
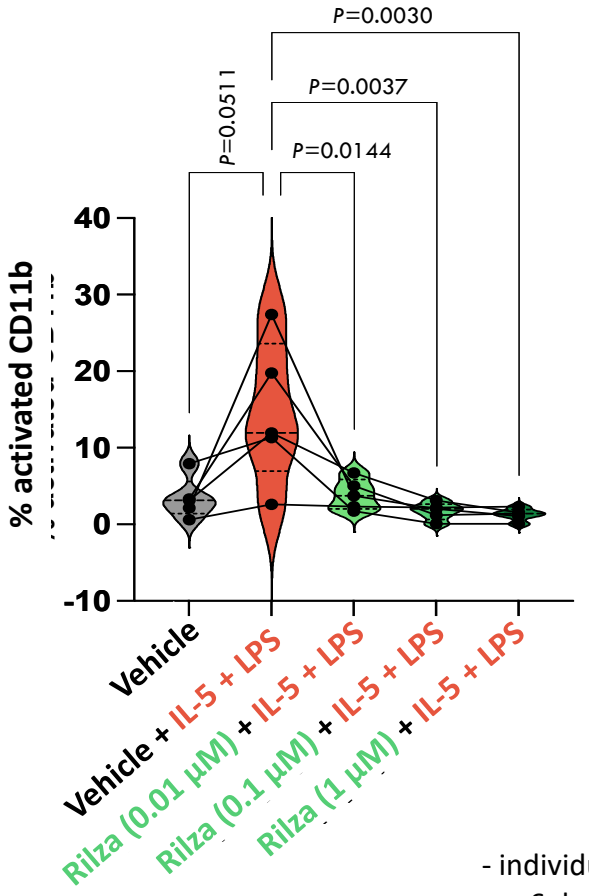


Flow cytometry analysis summary of CD11b activation



- individual donor
n = 6 donors

CD, cluster of differentiation; IL-5, interleukin 5; LPS, lipopolysaccharide; Rilza, rilzabrutinib.

Conclusion: BTK plays a novel role in human eosinophil activation and ROS generation elicited by IL-5 and LPS

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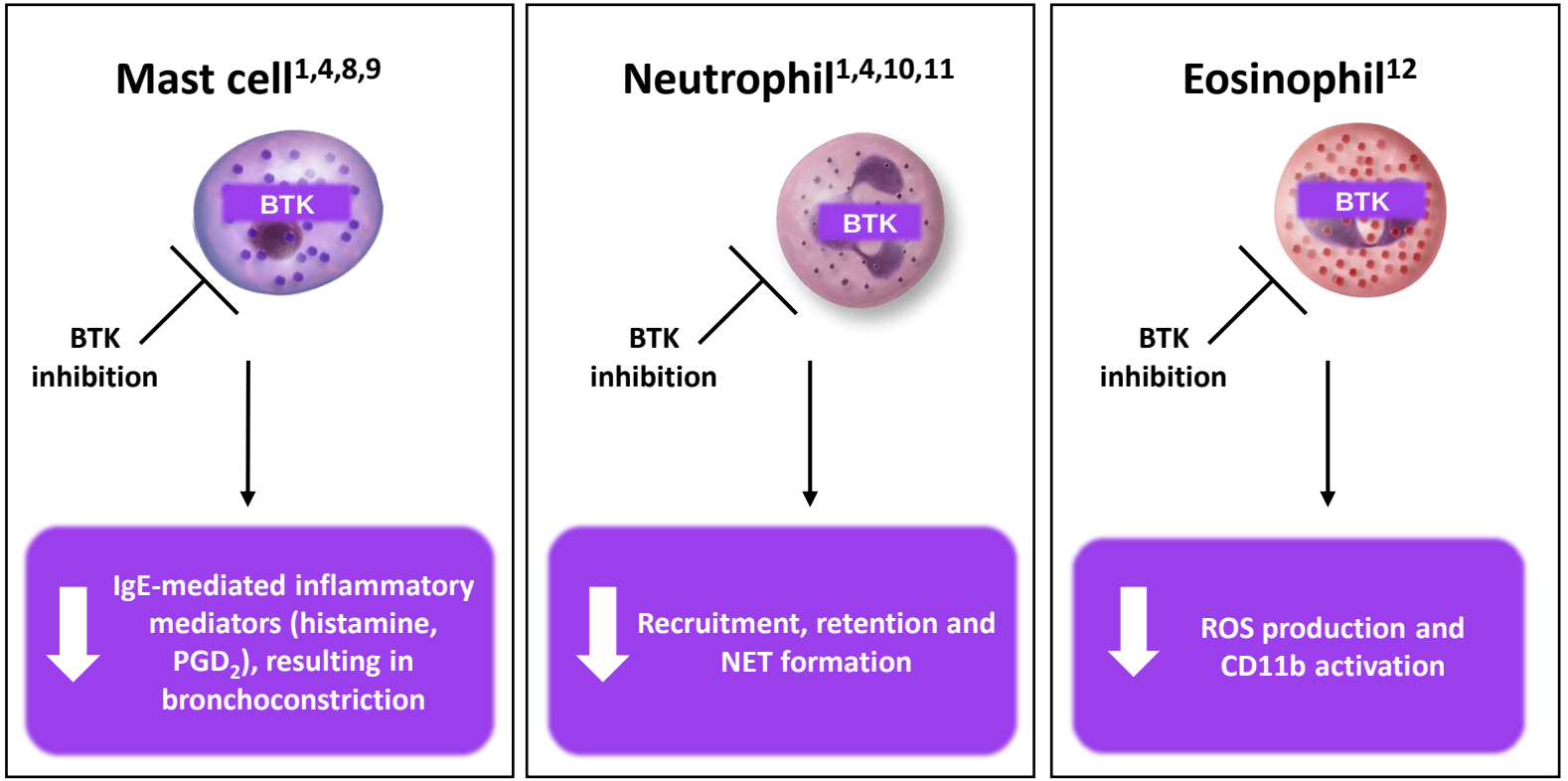
- Our studies demonstrate a novel role of BTK in human eosinophil activation and ROS generation
- Rilzabrutinib has the potential to reduce eosinophil-mediated tissue damage and chronic inflammation
- These findings provide preclinical support for the therapeutic potential of rilzabrutinib in treating inflammatory diseases, including asthma



BTK, Bruton's tyrosine kinase; CD, cluster of differentiation; IgE, immunoglobulin E; NET, neutrophil extracellular trap; PGD_2, prostaglandin D2; ROS, reactive oxygen species.

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