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Novel mechanisms of action contributing to benralizumab's potent anti-eosinophilic activity

Rania Dagher [1,4], Varsha Kumar[1], Alan M. Copenhaver[1], Sandra Gallagher[1], Mahboobe Ghaedi[1], Jonathan Boyd[2], Paul Newbold[3], Alison A. Humbles[1] and Roland Kolbeck[1,4]

[1]Bioscience COPD/IPF, Research and Early Development, Respiratory and Immunology, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA. [2]Imaging Core, Discovery Biosciences, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA. [3]Late Respiratory and Immunology, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA. [4]R. Dagher and R. Kolbeck contributed equally to this article as lead authors and supervised the work.

Corresponding author: Roland Kolbeck (rkolbeck@spirovant.com)

Shareable abstract (@ERSpublications)
New insights explaining the potent anti-eosinophilic activity of benralizumab, an anti-IL-5Rα afucosylated monoclonal antibody with enhanced depleting potency in patients with severe eosinophilic inflammation https://bit.ly/3yUnsnn

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Abstract

Background Benralizumab is a humanised, anti-interleukin-5 receptor α monoclonal antibody with anti-eosinophilic activity. Lack of fucose (afucosylation) increases its affinity to CD16a and significantly enhances antibody-dependent cell-mediated cytotoxicity by natural killer (NK) cells. Although benralizumab proved clinically efficacious in clinical trials for patients with severe asthma and hypereosinophilic syndrome, in-depth characterisation of its anti-eosinophilic mechanisms of action remains elusive.

Methods Here, we further investigated the mechanisms involved in benralizumab's anti-eosinophilic activities by employing relevant primary human autologous cell co-cultures and real-time-lapse imaging combined with flow cytometry.

Results In the presence of NK cells, benralizumab induced potent eosinophil apoptosis as demonstrated by the upstream induction of Caspase-3/7 and upregulation of cytochrome *c*. In addition, we uncovered a previously unrecognised mechanism whereby benralizumab can induce eosinophil phagocytosis/efferocytosis by macrophages, a process called antibody-dependent cellular phagocytosis. Using live cell imaging, we unravelled the stepwise processes leading to eosinophil apoptosis and uptake by activated macrophages. Through careful observations of cellular co-culture assays, we identified a novel role for macrophage-derived tumour necrosis factor (TNF) to further enhance benralizumab-mediated eosinophil apoptosis through activation of TNF receptor 1 on eosinophils. TNF-induced eosinophil apoptosis was associated with cytochrome *c* upregulation, mitochondrial membrane depolarisation and increased Caspase-3/7 activity. Moreover, activated NK cells were found to amplify this axis through the secretion of interferon-γ, subsequently driving TNF expression by macrophages.

Conclusions Our data provide deeper insights into the timely appearance of events leading to benralizumab-induced eosinophil apoptosis and suggest that additional mechanisms may contribute to the potent anti-eosinophilic activity of benralizumab *in vivo*. Importantly, afucosylation of benralizumab strongly enhanced its potency for all mechanisms investigated.