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Reply to correspondence of Elst *et al*

To the editor,

We thank Elst and colleagues for their commentary on our recently published article in this journal[1], which facilitates greater discussion on the topic. We agree that, although strongly suggested by pre-clinical studies, direct evidence for MRGPRX2 involvement in clinically-manifested, drug-induced anaphylaxis is currently wanting. The study of Navines-Fererr[2], that is sometimes used to suggest a direct role for MRGPRX2 in anaphylaxis to some neuromuscular blocking agents (NMBAs), is complex to interpret and careful, discriminatory characterisation between IgE and MRGPRX2 pathways in a clinical setting is challenging. The recent studies of Ebo *et al*[3,4], and the use of the basophil-activation test in clinical stratification, are thus a welcome addition to the literature. Importantly however, there remains a clinically significant cohort of patients where the IgE pathway, at least with the currently available diagnostic tests, does not explain the drug-induced anaphylaxis.

Clearly more research is needed to cement, or otherwise, the role of MRGPRX2 in drug-induced anaphylaxis. Whilst gain of function MRGPRX2 polymorphisms have been identified, the more common variants appear to be associated with reduced receptor activity and/or expression[5,6]. Standardised MRGPRX2 genotyping as part of studies examining drug-induced anaphylaxis would thus be of significant value. Moreover, MRGPRX2 receptor antagonists, perhaps developed around recent momentum in the contribution of MRGPRX2 to chronic inflammatory disease[7,8], might eventually also help unravel the contribution of this receptor to drug-induced anaphylaxis.

There is clear evidence for inter-species variability in agonist potency between human MRGPRX2 and its animal homologues, with the mouse form of the receptor (MrgprB2) being most commonly examined in this context. These results need to be considered somewhat carefully however as often higher-throughput, surrogate measures of mast cell functional responses (such as intracellular Ca^{2+}) are used and experiments conducted, for convenience, in commonly used cell lines that are not mast cells. These cell lines might not contain the appropriate signalling pathways present in mast cells that are responsible for functional amplification/signalling bias and a consequent true assessment of comparative potency. Crispr-Cas9 gene editing methods within a human mast cell context, as used in our study, might produce a more standardised set of comparative inter-species tools for such a

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purpose. Such an approach in an *in vivo* setting might also yield MRGPRX2 humanised mice that again would be a very valuable research tool[9].

Our study, like many others, failed to show any degranulation induced by rocuronium in LAD2 cells, and moreover complexes of sugammadex/rocuronium did not trigger degranulation (this data was not shown in our publication). Whilst we agree that we used a high concentration of sugammadex in our proof of concept studies, we also conducted more comprehensive concentration/response studies that are shown in the supplemental data section. This concentration range spans the plasma concentrations of sugammadex achieved clinically. However, we agree that any potential clinical inferences of the effect we observed of sugammadex on atracurium-induced MRGPRX2 activation are currently speculative and require further research.

We also agree that it is currently impossible for a clinician to discriminate between IgE and possible MRGPRX2-mediated anaphylaxis in an acute emergency setting. In many cases perioperative anaphylaxis to NMBAs are life-threatening events requiring immediate action. Early reports suggested sugammadex may attenuate this response but as noted, current consensus guidelines do not advocate the administration of sugammadex. The *in vitro* effects of sugammadex on MRGPRX2-dependent, human mast cell activation however has not been previously investigated, and in view of the critical nature of these cells in perioperative anaphylaxis, was the motivation for our study. That sugammadex inhibits MRGPRX2-mediated CCL-2 release, even when given some time following agonist addition, provides novel insight into the MRGPRX2 pathway and highlights use of sugammadex as a research tool. Our speculation around the possible clinical implications of these findings were raised to encourage consideration around the role of other mast cell mediators, asides from degranulation mediators, in longer-term symptoms of/recovery from anaphylaxis. Although, given the immediate medical emergency of anaphylaxis, the management of acute symptoms is of overwhelming priority.

MRGPRX2 activation in mast cells has emerged as a plausible mechanism to rationalise drug-induced anaphylaxis where evidence for involvement of IgE is lacking. However, further work is needed to establish the true importance of this receptor in this potentially devastating condition. If evidence is compelling, the question remains as to why only the few are susceptible to this reaction? This will be a key question likely answered in tandem with establishing the clinical importance of MRGPRX2. Given that around 20% of a drug-like compound library was recently shown to activate MRGPRX2[10], this is a critical question to be addressed for safe development of new drugs across the therapeutic spectrum.

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