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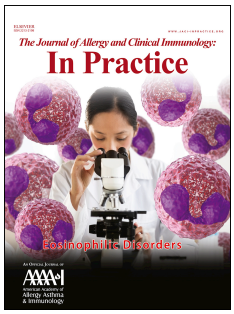


Endotyping of IgE-mediated polyethylene glycol and/or polysorbate 80 allergy.

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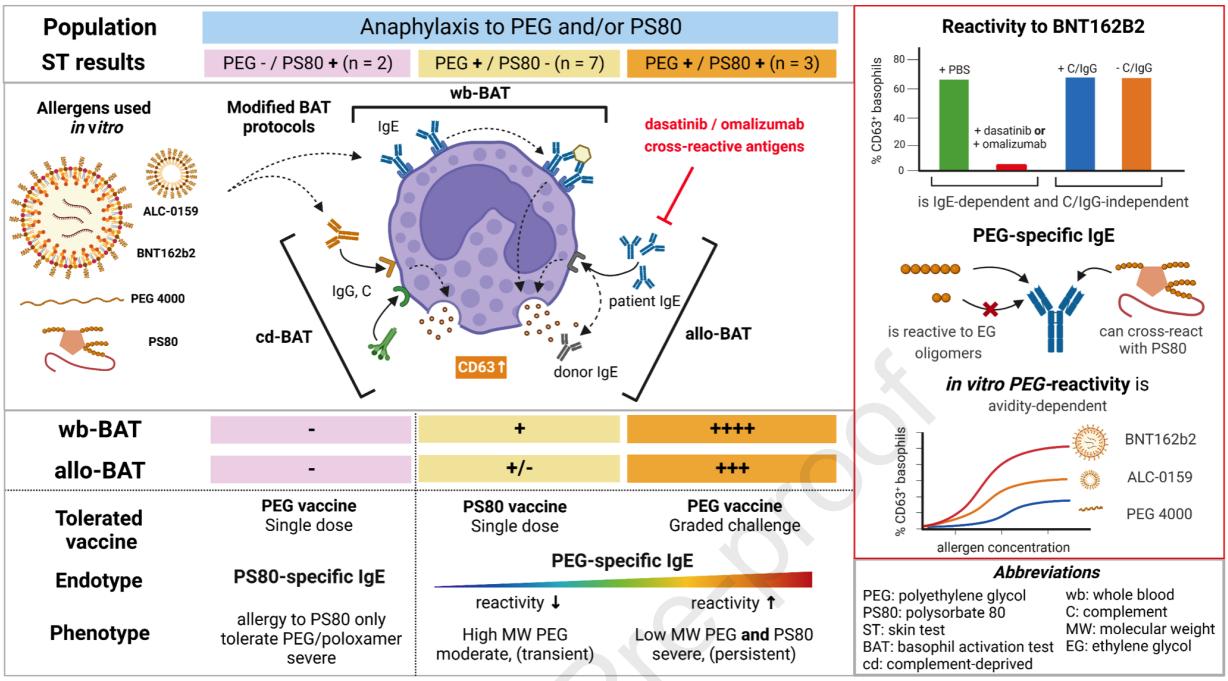
# Endotyping of IgE-mediated polyethylene glycol and/or polysorbate 80 allergy







***Abbreviations***

PEG: polyethylene glycol
PS80: polysorbate 80
ST: skin test
BAT: basophil activation test
cd: complement-deprived
wb: whole blood
C: complement
MW: molecular weight
EG: ethylene glycol

**Title:**

Endotyping of IgE-mediated polyethylene glycol and/or polysorbate 80 allergy.

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## Abstract

**Background:** Polyethylene glycol (PEG) and polysorbate 80 (PS80) allergy preclude from SARS-CoV-2 vaccination. The mechanism(s) governing cross-reactivity and PEG molecular weight-dependency remain unclear.

**Objectives:** To evaluate PEGylated lipid nanoparticle (LNP) vaccine (BNT162b2) tolerance, and explore the mechanism of reactivity in PEG and/or PS80 allergic patients.

**Methods:** PEG/PS80 dual- (n=3), PEG mono- (n=7) and PS80 mono-allergic patients (n=2) were included. Tolerability of graded vaccine challenges was assessed. Basophil activation testing on whole blood (wb-BAT) or passively sensitized donor basophils (allo-BAT) was performed using PEG, PS80, BNT162b2, and PEGylated lipids (ALC-0159). Serum PEG-specific IgE was measured in patients (n=10) and controls (n=15).

**Results:** Graded BNT162b2 challenge in dual- and PEG mono-allergic patients (n=3/group) was well-tolerated and induced anti-S IgG seroconversion. PS80 mono-allergic patients (n=2/2) tolerated single-dose BNT162b2 vaccination. Wb-BAT reactivity to PEG-containing antigens was observed in dual- (n=3/3) and PEG mono- (n=2/3), but absent in PS80 mono-allergic patients (n=0/2). BNT162b2 elicited the highest *in vitro* reactivity. BNT162b2 reactivity was IgE-mediated, complement-independent, and inhibited in allo-BAT by preincubation with short PEG motifs, or detergent-induced LNP degradation. PEG-specific IgE was only detectable in dual-allergic (n=3/3) and PEG mono-allergic (n=1/6) serum.

**Conclusion:** PEG and PS80 cross-reactivity is determined by IgE recognizing short PEG motifs, whilst PS80 mono-allergy is PEG-independent. PS80 skin test positivity in PEG allergics was associated with a severe and persistent phenotype, higher serum

PEG-specific IgE levels and enhanced BAT reactivity. Spherical PEG-exposure via LNP enhances BAT sensitivity through increased avidity. All PEG and/or PS80 excipient allergic patients can safely receive SARS-CoV-2 vaccines.

**Highlights box:**

***1. What is already known about this topic?***

The excipients PEG and PS80 are rare culprits of (multi-)drug allergies. Molecular weight-dependency favouring high molecular weight and cross-reactivity have been observed but remain poorly explained. Current guidelines contra-indicate SARS-CoV-2 vaccines in excipient allergic patients.

***2. What does this article add to our knowledge?***

PEG and PS80 cross-reactivity is determined by IgE recognizing short PEG motifs and represents a phenotypic extreme, clinically and mechanistically distinct from PS80 mono-allergy. Cross-reactive patients tolerate PEG-containing vaccines through graded challenge.

***3. How does this study impact current management guidelines?***

An allergist-guided approach enables SARS-CoV-2 vaccination in all PEG and/or PS80 allergic patients. PS80 sensitization in PEG allergic patients could be a biomarker for more severe allergy phenotypes. PS80 mono-sensitization is a distinct phenotype lacking cross-reactivity with PEG.

**Keywords:**

basophil activation test, polyethylene glycol, polysorbate 80, BNT162b2, ALC-0159, SARS-CoV-2, vaccine, allergy, IgE, cross-reactivity

## Abbreviations

- AC: anaphylaxis control
- BAT: basophil activation testing
- CARPA: complement activation-related pseudoallergy
- cd-BAT: complement-deprived basophil activation test
- CDC: Centers for Disease Control and Prevention
- DA: dual-allergy
- DEG: diethylene glycol
- EG: ethylene glycol
- GVC: graded vaccine challenge
- HC: healthy control
- HDM: house dust mite
- HMW: high molecular weight
- LMW: low molecular weight
- LNP: lipid nanoparticle
- MW: molecular weight
- PEG: polyethylene glycol
- PS80: polysorbate 80
- SEM: standard error of mean
- sIgE: specific IgE
- ST: skin test
- tIgE: total IgE
- wb-BAT: whole blood basophil activation test

## INTRODUCTION

Polyethylene glycols (PEGs) or macrogols are formed through polymerisation of ethylene oxide, resulting in linear chains of varying numbers of ethylene glycol (EG) subunits.[1,2] The PEG number equals the molecular weight (MW) and correlates with the number of EG subunits. As nonionic surfactants with low toxicity, PEGs are ubiquitously used as excipients or additives in a wide range of biotechnological applications. Polysorbate or Tween 80 (PS80) is a structurally related nonionic surfactant consisting of an oleic acid tail and sorbitane core with 4 sidechains, each equivalent to a PEG 220 moiety (**Fig E1** in this article's **online repository**).[3,4] IgE-mediated allergy to PEG and/or PS80 is ultra-rare but has important clinical implications due to its potential severity, wide range of potential triggers and challenges in avoiding inadvertent exposure.[5] Diagnosis is based on a history of immediate reactions upon exposure and demonstration of sensitization through skin testing (ST) with high specificity but comparatively low sensitivity.[4] In addition, ST reactivity to PEGs can wane over time.[4] Many clinical observations in PEG and/or PS80 allergic patients can be considered unique and remain unexplained from a mechanistical point-of-view. Firstly, most PEG allergic patients only react to high MW (HMW) PEGs.[6,7] Secondly, ST reactivity wanes over time but can remain for HMW PEGs.[4] Thirdly, although cross-reactivity is not always reported, two case series indicated demonstrable ST cross-reactivity with PS80 in around 30% of PEG allergic patients.[3,4] Lastly, ultra-rare PS80 allergy without obvious cross-reactivity to PEG has also been observed, yet remains mechanistically unexplained.[3,8,9]

Excipient allergies have gained attention in the context of the global COVID-19 vaccination campaign. All currently available SARS-CoV-2 vaccines in the United States and European Union contain either PEG 2000 (BNT162b2, Pfizer/BioNTech;

mRNA-1273, Moderna) or PS80 (Ad26.COV2.S, Janssen; AZD1222, Oxford/AstraZeneca; NVX-CoV2373, Novavax; VidPrevtyn Bèta, Sanofi Pasteur).[10,11] The lipid nanoparticles (LNP) of the mRNA-based BNT162b2 and mRNA-1273 vaccines contain PEG in the form of PEGylated lipids. These lipids form part of the LNP envelope and consist of hydrophobic fatty acid tails covalently linked to PEG 2000 moieties which protrude from the LNP's globular surface, forming a protective hydrophilic coating (**Fig E1**).[12] Post-marketing surveillance signalled a risk for anaphylaxis with mRNA-based SARS-CoV-2 vaccines of around 5 per million doses.[13,14] Several mechanisms have been proposed, including complement activation-related pseudoallergy (CARPA), IgG-mediated anaphylaxis, direct non-allergic mast cell activation and classic type I IgE-mediated allergy.[15-18] PEG and PS80 have been implicated as potential triggers but their role remains controversial.[19-22] Current guidelines issued by the Centers for Disease Control and Prevention (CDC) contra-indicate the use of SARS-CoV-2 vaccines in patients with pre-existing excipient allergies.[23] While we and others have demonstrated that patients with PEG and PS80 mono-allergy may safely receive vaccines containing the alternative excipient, this still precludes patients with sensitization to both PEG and PS80 from SARS-CoV-2 vaccination.[3,24]

In this study we investigated tolerance of graded PEG-containing BNT162b2 vaccine challenges in 3 patients with ST-confirmed sensitization to PEG and PS80. Secondly, patients reacting to both PEG and PS80, or only PEG or PS80 provided a unique disease model to disentangle the mechanisms behind PEG and PS80 cross-reactivity, the observed PEG MW-dependency, and *in vitro* reactivity to PEG-containing products.

## METHODS

### Patient selection and sampling

We included patients with sensitization to both PEG and PS80, hereafter termed dual-allergic (DA, n=3), or sensitization to only PEG (n=7) or PS80 (n=2), termed mono-allergic, diagnosed at our department between 2009 and 2021 (see **Table I** and the **online repository** for detailed patient characteristics). Diagnosis was based on a combination of clinical history and ST. DA1 and DA2 were diagnosed prior to the COVID-19 pandemic and underwent ST for PS80 prior to SARS-CoV-2 vaccination whereas DA3 was referred due to reactions possibly related to PEG allergy and the need for SARS-CoV-2 vaccination. All DA patients underwent in-hospital vaccination with BNT162b2 through graded vaccine challenges (GVC), according to a protocol adapted by Huyhn *et al.* from the 2012 practice parameter update of the AAAAI/ACAAI joint task force on adverse reactions to vaccines (see **Fig 2** and **Table E1**).[25,26] All PEG and PS80 mono-allergic patients were initially referred to our department outside of the context of SARS-CoV-2 vaccination. Blood samples were obtained at different timepoints (**Table E2**) in accordance with a prospective study protocol on rare causes of anaphylaxis approved by the Ethics Committee Research UZ/KULeuven (reference number S60734, see **online repository** for additional info). Additional biobanked serum samples for measurement of PEG-specific IgE (sIgE) were obtained from patients within the same study with a history of non-PEG/PS80-related anaphylaxis (anaphylaxis controls, AC, n=15, **Table E4**). Healthy controls (HC, n=6) were recruited among non-allergic volunteers with a history of tolerated exposure to BNT162b2 (**Table E5**). All participants provided written informed consent prior to sampling.

### *In vitro* assays

Blood samples were used for basophil activation testing (BAT) and measurement of serum tryptase, total IgE (tIgE) and sIgE towards PEG 2000 and 10,000 as well as SARS-CoV-2 specific IgG (see **online repository** for detailed methods). For whole blood BAT (wb-BAT), fresh blood samples were incubated at 37°C for 25 minutes with various stimuli and controls followed by lysing, washing, staining with fluorochrome-conjugated antibodies (anti-CD63, anti-HLA-DR and anti-CD123) and fixation in paraformaldehyde. Processed samples were acquired on an LSRFortessa flowcytometer equipped with FACSDiva software and analyzed using FlowJo v10.8.1 (BD, San Jose CA, USA). Basophils were gated as $CD123^{+}$/$HLA\text{-}DR^{-}$ cells and CD63-expression was used as a surrogate marker for basophil degranulation (see **Fig E2** for gating strategy).[27] The reagents used are listed in **Table E6**. In addition, modified BAT assays termed complement-deprived BAT (cd-BAT) and allo-BAT were performed, outlined in detail in the **online repository**.[28-30] In addition, allo-BAT was performed in the presence of inhibitors of IgE-dependent signalling including dasatinib and omalizumab (see **online repository and Fig E7** for details).[31]

Statistics

Statistics were performed using GraphPad Prism, version 9.3.1 (GraphPad Software, San Diego CA, USA). Data is shown as either individual datapoints or mean + standard error of mean (SEM). Groups were compared using a paired samples t-test or Wilcoxon matched-pairs signed-rank test where appropriate. All tests were performed with a two-sided significance level of 0.05. Illustrations were created using BioRender (Toronto, Ont., Canada).

## RESULTS

Phenotyping distinguishes three excipient allergic groups, all tolerating BNT162b2 vaccination

Twelve patients with ST-confirmed PEG and/or PS80 allergy were included (see **Fig 1** for clinical timeline). Seven patients (58%) were female and median age at diagnosis was 46 years (range 19-71). Median baseline tryptase was 4.8 µg/L (range 2.4-9.8) and median total IgE was 113 kU/L (range 22-3403). Individual clinical and biochemical characteristics are outlined in **Table I** and described in detail the **online repository** (clinical vignettes, **Table E2-E3**, including differential blood counts, CRP, total Ig levels and sIgE towards common aero-allergens). Three groups could be distinguished.

Group 1 included DA patients (n=3; 1/3 female, median age 23y). All demonstrated ST reactivity to both PEG and PS80 and had multiple, often severe reactions (anaphylaxis with hypotension) after exposure to both low MW (LMW, < 1000 Da) and HMW PEGs and upon systemic as well as topical exposure, i.e. PEG-containing cosmetic products. DA3 also reacted to a PS80-containing drug (Cordarone®). All DA patients received and tolerated BNT162b2 through GVC on at least 2 occasions (**Fig 2, A**). No transient tryptase elevation was observed during the GVC protocols (**Fig 2, B**). All DA patients were SARS-CoV-2 naive and demonstrated adequate vaccine-induced anti-S IgG seroconversion (**Fig 2, C**).[32] Based on positive repeat ST results as well as occurrence of multiple clinical reactions in the interval between diagnosis and vaccination, DA patients exhibited persistent clinical reactivity throughout the 4-11 year follow-up period (**Fig 1**).

Group 2, were PEG mono-allergic (n=7; 4/7 female, median age 46y), exhibiting clinical and ST reactivity to PEG only. Severe events (i.e. anaphylaxis with hypotension) were

noted in only 2 patients with the remaining 5 reporting generalized urticaria with associated minor gastro-intestinal and/or respiratory symptoms. All reacted upon exposure to intra-articular corticosteroid injections with only 2/7 (PEG5,7) reporting multiple reactions. All reactions occurred upon parenteral exposure to HMW PEGs (≥ 3350 Da). All PEG mono-allergics were invited for additional PS80 ST prior to vaccination, except in 2 cases where PS80 tolerance had been demonstrated earlier (PEG6,7). The remaining 5 had negative PS80 ST and subsequently tolerated single-dose administrations of PS80-containing vaccines, as previously reported.[3] Three patients (PEG1,6,7) also tolerated a subsequent GVC with BNT162b2. Of particular note, repeat PEG ST prior to vaccination were negative in 3/3 patients where this was performed (PEG1,2,4) and one patient (PEG6) reported tolerated exposure to an oral PEG 4000-containing bowel preparation prior to the fourth vaccine dose (**Table E2**). In contrast with group 1, apparent loss of clinical reactivity throughout the 5-13 year follow-up period was observed. This loss of reactivity to PEG was deemed to be highly likely in at least 4/7 and possible in the remaining 3/7 subjects (**Fig 1**). Therefore, these patients are hereafter referred to as 'previously PEG mono-allergic'.

Group 3, termed PS80 mono-allergic, had clinical and ST reactivity to PS80 only (n=2, PS1,2; 2/2 female, aged 46 and 50y). Both had severe reactions (anaphylaxis with hypotension) upon parenteral exposure to PS80-containing drugs, including a PS80-containing SARS-CoV-2 vaccine (Vaxzevria®) and rituximab (PS2). PS2 afterwards tolerated intravenous administration of a PS80-free anti-CD20 monoclonal antibody (obinutuzumab, Gazyvaro®) containing the PEG-based excipient poloxamer 188 (**Fig E1**) and both patients tolerated multiple single-dose PEG-containing BNT162b2 vaccines (**Fig 1**).

<u>Basophil reactivity to PEG-based antigens is highest in DA patients</u>

*In vitro* wb-BAT reactivity to PEG-containing compounds (BNT162b2, ALC-0159, PEG 4000, PEG 20,000, PS80) was assessed in DA patients (n=3/3), immediately before and after GVC, and in previously PEG mono-allergics (n=3/7; PEG1,6,7), PS80 mono-allergics (n=2/2), and concurrent HC (n=6) (**Fig 3, A**; **Fig E3**; **Table E7**).

DA basophils exhibited strong dose-dependent reactivity to BNT162b2 (80.9-95.7% CD63 expression) which remained stable throughout the study period (**Fig 3, B-C**). PEG 4000 and PS80 tested positive in 2/3 DA patients and induced lower reactivity (16.7-32.3% and 12.5-13.6%, respectively) which disappeared in DA3 for PEG 4000 at the second GVC (**Fig E3**). Reactivity was even lower to PS80, and absent to PEG 20,000 (tested in DA3 only). The PEGylated vaccine lipid, ALC-0159, tested at the 7wASD timepoint, elicited a response in 1/2 tested patients (DA3, 28%).

Basophils of previously PEG mono-allergic patients (n=3/7 with available fresh samples, **Table E2**) also exhibited reactivity to BNT162b2, albeit less pronounced (17.2-76.1%) with only 1 patient (PEG1) exhibiting reactivity to PEG 4000. No reactivity to ALC-0159 or PS80 was observed in this group (**Fig E4**).

None of the two PS80 mono-allergic patients demonstrated *in vitro* reactivity to the tested antigens, although PS2 was IgE non-responder (< 10% CD63-expression with anti-IgE).

All antigens were concurrently tested in fresh blood samples of non-allergic HC including 3 with a PCR-confirmed SARS-CoV-2 infection in the 3 months prior to sampling (**Table E5**). None exhibited reactivity to BNT162b2 (n=0/6), ALC-0159 (n=0/6), PEG 20,000 (n=0/2), PEG 4000 (n=0/3) or PS80 (n=0/3).

The overall lower (~50%) allo-BAT responses compared to wb-BAT were considered assay- and not stimulus-dependent (**Fig E5, B**). A positive allo-BAT was observed in

all (3/3) DA patients using BNT162b2 and ALC-0159, whilst only, to a lesser degree, in 1/7 and 3/7 previously PEG mono-allergic patients, respectively (**Fig 4, A**; **Table E8**). PEG 4000 and PS80 could not elicit allo-BAT reactivity in any of the subjects (data not shown).

Median time between index reaction and BAT was 10 years in DA patients (range 4-11y, n=3), 7 years and 4 months in previously PEG mono-allergics (range 4y7m-13y, n=7) and 9.5 months in PS80 mono-allergics (range 9-10m, n=2) (**Table E2**). Despite similar intervals between index reaction and *in vitro* testing, wb-BAT (**Fig 2**) and allo-BAT (**Fig 4, A**) reactivity to PEG-based antigens was stronger in the DA subgroup *vs.* previously PEG mono-allergics, correlating with persistence of clinical reactivity.

Basophil reactivity in PEG allergic patients is IgE-mediated

Allo-BAT was used to confirm IgE-dependence of the observed reactivity. Reactivity to BNT162b2 and ALC-0159 could be transferred to non-allergic basophils using serum from DA, and to a lesser extent, previously PEG mono-allergics, but not PS80 mono-allergics or HC (**Fig 4, A**).

Heat-inactivation abrogated this transfer and complement add-back through unaltered HC serum prior to stimulation did not restore reactivity, indicating a role for PEG-sIgE in patient serum (**Fig E5, A**). Next, we excluded classic (through PEG-IgG/IgM immune complexes) or alternative complement pathway activation (through PEGylated LNPs) with C3a and C5a-induced basophil activation, as hypothesized earlier.[16,17,33] We performed a complement-deprived BAT (cd-BAT) with available fresh blood samples of BNT162b2 responders (n=4 samples, DA2-3, PEG1). After removal of the complement-containing plasma fraction, cells were resuspended in either unaltered or heat-inactivated serum of a non-allergic control (HC5) prior to stimulation (**Fig 4, B**).

Both conditions yielded similar dose-responses after stimulation with BNT162b2, comparable to wb-BAT responses. Lastly, allo-BAT reactivity was assessed using sera of DA patients (DA1-3) after basophil pretreatment with dasatinib or DA serum preincubation with omalizumab. Both conditions abrogated responses to PEG-based antigens (BNT162b2, ALC-0159) and house dust mite (HDM) extract (DA2 only) (**Fig 4, C**), confirming that IgE-cross-linking by BNT162b2 and ALC-0159 was responsible for the observed reactivity.

PEG-specific IgE can cross-react with PS80 in DA patients.

To assess epitope-specificity of this IgE, we evaluated basophil reactivity to PEG-based antigens through serum preincubation with various potentially cross-reactive antigens (**Fig 5, A**). Preincubation with PEG 4000 (90 EG subunits), PEG 400 (9 EG subunits), as well as PS80 (4 sidechains with 5 EG subunits each), but not diethylene glycol (DEG, 2 EG subunits, **Fig E1**), abrogated allo-BAT reactivity to BNT162b2 and ALC-0159. Preincubation did not abrogate responses to control stimuli (anti-IgE, fMLP) or HDM (in DA2). To further delineate titer-dependence, allo-BAT sensitization was performed with serial dilutions of DA and previously PEG mono-allergic sera (**Fig 5, B**). DA sera exhibited higher baseline reactivity in allo-BAT as well as resistance to dilution before losing the ability to transfer BNT162b2 and ALC-0159 reactivity compared to previously PEG mono-allergic sera.

Spherical presentation of PEG facilities *in vitro* BAT reactivity

We hypothesized that PEGylated LNP induced more PEG-sIgE cross-linking due to increased density and spherical presentation of PEG epitopes (**Fig E1**). Analysis of ALC-0159 dose-responses revealed maximal basophil activation around its critical micellar concentration (**Fig 5, C**).[34,35] We next assessed BAT reactivity after disruption

of LNP integrity using a zwitterionic detergent (CHAPS). At a non-toxic CHAPS concentration (0.5%), allo-BAT reactivity to HDM was unaltered (DA2) but reactivity to BNT162b2 was abolished in all DA sera (**Fig 5, D**). Since PEG and PS80 are nonionic surfactants, we verified whether their inhibitory effects on BNT162b2 reactivity in DA sera was dependent on direct detergent action. When serum and excipients were washed away after sensitization and prior to stimulation, residual inhibition consistent with sequestration of PEG-sIgE and partial detergent action was observed (**Fig E6**).

PEG sIgE is measurable in DA patients

Specific IgE towards PEG 2000 and PEG 10,000 was quantified in serum of PEG and/or PS80 allergic patients, HC, and patients with a history of non-PEG-related anaphylaxis, tolerance to BNT162b2, and varying tIgE (between 22 and >5000 kU/L; anaphylaxis controls, AC; n=15; see **Table E4** for additional information) (**Fig 6**; **Fig E8**; **Table E9**). PEG 2000 and PEG 10,000 sIgE was detectable (>0.35 kU/L) in all DA patients (3/3; 0.94 to >100 kU/L) and 1/6 previously PEG mono-allergic patients (PEG5; 0.7 kU/L) but not in PS2, HC (0/6) or AC (0/15) (**Fig 6, A**). PEG1 and PEG6 lacked detectable PEG-sIgE on ImmunoCAP despite positive wb-BAT with BNT162b2 and/or PEG 4000. Increasing PEG-sIgE fluorescence signal was noted in HC and AC when tIgE increased above 300 kU/L, resulting in PEG-sIgE values > 0.1 kU/L but < 0.35 kU/L in all samples with tIgE above 3000 kU/L (**Fig 6, B**). Cross-reactivity between PEG-sIgE and BNT162b2 was assessed by preincubating DA (n=3) and AC sera (n=6) with undiluted BNT162b2 or PBS at 10% v/v. All preincubated DA sera showed partial PEG-sIgE inhibition whereas non-specific reactivity in high tIgE AC sera remained unaffected (**Fig 6, C**; **Fig E8, B**).

## DISCUSSION

In this work, we demonstrate the **tolerability of GVC with a PEG-containing** COVID-19 vaccine in PEG and PS80 cross-reactive patients, previously excluded from vaccination. Combined with previous findings, this demonstrates feasibility of COVID-19 vaccination in all patients with rare PEG and/or PS80 allergy.[3,24] Through **clinical and *in vitro* evaluation, we infer**: a) 3 possible endotypes of IgE-mediated excipient allergy (DA, PEG mono-, and PS80 mono-allergics respectively); b) PEG and PS80 cross-reactivity in DA patients due to recognition of small PEG oligomers (of 3-5 EG subunits); and c) avidity and 3D presentation explaining the stronger *in vitro* reactivity to PEGylated LNP compared to linear PEG and the observed MW-dependency.

Within the **proposed endotypes** (**Fig 7**), patients with sensitization to both PEG and PS80 (designated as DA in this article) exhibited the most severe and persistent clinical phenotype, reacting to parenteral up to topical exposure and high up to lower MW PEGs (i.e. PEG 400 or PS80). They also showed stronger *in vitro* reactivity in BAT and sIgE assays. PEG mono-allergics had a milder and apparently transient phenotype, reacting mostly to parenteral exposure and to higher MW PEG-containing antigens. *In vitro* reactivity to PEG-based compounds was only observed in a subset of these patients. Finally, PS80 mono-allergy was associated with severe reactions upon parenteral exposure to PS80, but tolerance to PEG-based compounds (e.g. single-dose BNT162b2 and parenteral poloxamer 188). We theorize that recognition of a non-PEG-based epitope in PS80 could explain this observation. Given the limited cohort, we anticipate that additional subgroups can be identified. Based on our findings, we hypothesize that PS80 sensitization could be a biomarker for the most severe and persistent subgroup of PEG allergy and suggest including (non-irritant) PS80 ST in the diagnostic workup of excipient allergy to allow validation of this hypothesis. Previous

work indicated **waning ST reactivity** to PEG, with persistent ST positivity mainly observed for higher MW PEGs.[4] This was also observed in some of our previously PEG mono-allergic, but not in DA patients. Waning reactivity in a subset of patients may have also influenced the endotype-phenotype associations we observed, as these might be different when assessed at initial presentation. Regardless, a combination of persistent ST and *in vitro* reactivity as seen in our DA subgroup seems to correlate well with the presence of active and severe PEG allergy. Whether waning ST and/or absent *in vitro* reactivity as seen in the previously PEG mono-allergic subgroup is accompanied with a loss in clinical reactivity to PEG in all cases cannot be definitively concluded from our data, but is conceivable and merits further validation.

All DA patients and a subset of previously PEG mono-allergics **exhibited *in vitro* reactivity to BNT162b2 despite *in vivo* tolerability,** albeit to fractioned administration. Potential explanations include: a) desensitization through the GVC protocol, b) PEG doses in the administered intramuscular vaccine fractions not reaching the threshold for clinical reactivity, or c) *a priori* lack of clinical reactivity to the vaccine in PEG allergics. The absence of changes in CD63-based BAT responses early after GVC is likely unable to rule out actual desensitization, as reported earlier in rapid drug or venom desensitization studies.[36-39] Some reports have demonstrated tolerability of single-dose PEG-containing vaccines in a limited number of patients with recent ST-confirmed PEG allergy.[20,22] However, PS80 sensitization was not uniformly reported in these studies, hampering comparison with our cohort. Waning reactivity to PEG might also play a role. Our DA and PEG patients were not exposed to single-dose PEG vaccines and potential clinical reactivity to single-dose administration is uncertain. On the other hand, several cases of PEG allergy diagnosed after SARS-CoV-2 vaccine-related anaphylaxis were described, suggesting that PEG allergy could

predispose towards mRNA vaccine anaphylaxis.[40] Regardless, our findings underscore the safety and feasibility of an easy-to-perform GVC in these ultra-rare patients. **Our current approach** in case of a known or suspected PEG and/or PS80 allergy, is to first define the subgroup using ST and *in vitro* assays, when available (i.e. BAT with PEG-based LNP and PEG-sIgE measurement). Next, we advise single-dose PS80 in PEG mono-allergics and PEG-containing vaccines in PS80 mono-allergics, and in-hospital GVC with PEG-containing vaccines in DA patients.[3,24] The latter can also be an option for PEG mono-allergics in case of limited PS80-based vaccine availability or hesitancy (**Fig 7**).

We identified **BAT with PEGylated LNP as a marker for IgE sensitization to PEG** which could be used in addition to ST and PEG-sIgE measurement to guide clinical decisions on PEG-based drug administrations (e.g. vaccines, or other). However, as demonstrated in other settings, BAT does not distinguish perfectly between allergy and sensitization.[41,42] Given the limited number of cases and exclusive use of CD63 as single activation marker, determination of sensitivity and specificity of BAT in PEG allergy as well as its value in predicting clinical reactivity, which would require concurrent single-dose provocations, fell outside our scope. In addition to BAT, PEG-sIgE determination through ImmunoCAP also holds promise for implementation in clinical practice as it appears to be a specific diagnostic tool, especially when accounting for high tIgE.

**Increased avidity** likely explains the stronger *in vitro* basophil responses with spherical PEGylated LNP compared to unincorporated linear excipients, as proposed earlier.[6,43-45] This could be exploited to improve BAT sensitivity, in line with studies using artificial high-avidity allergen constructs with PEGylated nanoparticles, dendrimeric beta-lactams and aeroallergen-coated gold particles.[44,46,47] The potential

445 *in vivo* relevance is underscored by scarce reports of PEG-related mRNA vaccine
446 reactions and IgE-dependent anaphylaxis to liposomal PEGylated echocardiography
447 contrast.[40,48] This concept could also explain why PEG allergics typically react to HMW
448 PEG as these contain more potential epitopes. Similarly, it would explain why clinically
449 relevant cross-linking by LMW (low-avidity) PEG, including PS80 at the low end of the
450 spectrum, requires sufficiently reactive (i.e. high titer and/or affinity) PEG-sIgE.
451 Contrary to previous studies illustrating the sensitivity of **PEG 20,000** ST in PEG allergy
452 diagnosis, we could not demonstrate BAT reactivity to linear PEG 20,000.[4-7] Some
453 patients did react to linear PEG (2000, 4000) on BAT or had demonstrable sIgE
454 towards PEG (2000, 10,000) on ImmunoCAP yet routine skin and *in vitro* testing with
455 PEG 20,000 was not performed in all patients at initial diagnosis but only added at a
456 later stage in our study in a small subset of patients. Mechanistic differences between
457 *in vitro* BAT and *in vivo* mast cell or basophil activation by longer linear PEGs could
458 play a role. Additionally, since linear PEGs exhibit significant conformational flexibility
459 in aqueous media, PEG lengths exceeding a certain threshold might paradoxically
460 reduce IgE-cross-linking ability *in vitro* through reduced effective avidity and/or steric
461 hindrance at the basophil surface.[49-51] These characteristics might affect the partial
462 discordance in outcomes of epitope-paratope binding assays (i.e. ImmunoCAP) *vs.*
463 cross-linking assays (i.e. BAT). The determinants of PEG-IgE binding and cross-linking
464 *in vitro* and *in vivo* remain an important topic for future research.

465 **Limitations of our work included**: a) small sample size and b) partly retrospective
466 design, resulting in a lack of standardized ST at initial diagnosis and upon retesting,
467 varying sampling intervals, and only partial availability of fresh samples for full *ex vivo*
468 analysis, both of which may have biased the interpretation of possible endotype-
469 phenotype associations; c) no head-to-head comparison of single-dose *vs.* graded

PEG-based vaccine tolerability, given the clinical need and prevailing guidelines at the time;[25] d) inability of BAT and ImmunoCAP to disentangle IgE titer and affinity;[52] and e) exclusive reliance on CD63 as sole activation marker. Our study also did not aim to explain SARS-CoV-2 vaccine-related anaphylaxis, as almost no cases follow the classical paradigm of IgE-mediated allergy, with excipient allergy representing an exception rather than the rule.[22,53,54] Our pilot study warrants validation in larger prospective studies including longitudinal *in vivo* and *in vitro* workup with a full spectrum of PEG-based excipients in a larger cohort of patients, starting at a timepoint as close as possible to the initial index reaction. Future work would preferentially also include CD203c, histamine, and/or mast cell activation assays to further delineate effector cell activation. In addition, future studies should also focus on the potential impact of inherited and acquired genetic modulators of allergy severity (i.e. hereditary alpha-tryptasemia, somatic KIT mutations) on the observed phenotypes of PEG allergy and drug allergy in general.[55]

**In summary**, our findings support a novel endotype-phenotype hypothesis for IgE-mediated PEG and/or PS80 allergy and indicate that strong *in vitro* reactivity to PEGylated LNP and PS80 ST reactivity could be biomarkers for severe and persistent IgE-mediated PEG allergy. We demonstrate that all excipient allergic patients, including those with sensitization to both PEG and PS80, can safely receive (allergist-guided) SARS-CoV-2 vaccines. Prospective multicenter studies to validate our proposed endotypes and clinical workup are highly anticipated.

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**Author contributions:**

TI, LC, DB and RS designed experiments. TI and LC performed experiments. MV, ST, HV, LS, CB and RS provided clinical diagnostic work-ups and graded vaccine challenges. GF and PP provided laboratory support. DD provided pharmaceutical support. DW assisted with sample selection and clinical data extraction. TI and RS analyzed the data and wrote the manuscript. DB and RS supervised the study. All authors reviewed and revised the manuscript.

**Conflict of interest statement:**

All authors declare that they have no conflicts of interest to disclose.

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692

693 **Figure legends:**

694 **FIG 1.** Clinical timeline of excipient allergy. Horizontal bars indicate longitudinal allergy
695 course in individual patients starting at the index reaction ($R_{index}$, X=0) up to the last
696 administered SARS-CoV-2 vaccine dose. Vertical lines indicate clinical milestones i.e.
697 excipient-related allergic reactions (red lines), tolerated excipient exposure (green
698 lines), excipient skin testing and administered SARS-CoV-2 vaccines (black lines).
699 Exposure and skin test outcomes are indicated in either red (reactive/positive) or green
700 (tolerated/negative). Confirmed reactivity (pink bars) was inferred in patients who
701 experienced additional clinical reactions after diagnosis and/or in case of persistent
702 positive skin tests for the causal and/or cross-reactive excipients. Uncertain reactivity
703 (orange bars) was inferred in absence of repeat exposure to or skin testing with the
704 causal excipient after initial diagnosis. Possible tolerance (grey bars) was inferred in
705 PEG allergics if subsequent PS80 skin testing or exposure were negative. Likely
706 tolerance (green bars) was inferred if repeat skin testing with the causal excipient
707 became negative and/or tolerated re-exposure could be ascertained. See also clinical
708 vignettes in the **online repository**.

709 *Abbreviations:* PEG, polyethylene glycol; PS80, polysorbate 80; ST, skin test; GVC,
710 graded vaccine challenge with BNT162b2; SD, single-dose SARS-CoV-2 vaccine
711 administration.

712 **Suggested figure width:** 2 columns

713 **FIG 2. A**, graded vaccine challenge protocol with BNT162b2 performed in dual-allergic
714 patients (DA1-3). Median time between last PEG-related reaction was 36 months
715 (range 5 months – 10 years). Five consecutive vaccine dose fractions (D1- 5),
716 indicated as percentage of total standard dose, were administered at 15 minute
717 intervals over a 1 hour period. Arrows indicate timepoints of blood sampling: BFD,
718 before first dose; AFD, after first dose; BSD, before second dose; 7wASD, 7 weeks
719 after second dose (DA2 and 3 only). BAT, basophil activation test. **B**, paired serum
720 tryptase analyses for each patient (DA1-3) immediately before (closed symbols) and 1
721 hour after (open symbols) each vaccine challenge. FD, first dose; SD, second dose.
722 **C**, evolution of anti-SARS-CoV-2 spike IgG (anti-S) and anti-nucleocapsid IgG (anti-N)
723 during the vaccination protocol. Dotted line: reporting limit of anti-S IgG assay (21
724 AU/mL) or manufacturer's cutoff for anti-N assay positivity (S/CO > 1.40). AU, arbitrary
725 units; S/CO, signal-to-cutoff ratio.

726 **Suggested figure width:** 2 columns

727

**FIG 3.** Whole blood basophil activation test (BAT) responses (% $CD63^+$ basophils) in PEG and/or PS80 allergic patients and controls. **A**, baseline wb-BAT responses to positive controls and PEG or PS80-based antigens in healthy controls (green triangles, n=6), dual-allergic patients (closed squares, n=3), previously PEG mono-allergic patients (open squares, n=3), and PS80 mono-allergic patients (blue circles, n=2; PS2 was non-responder to anti-IgE). ALC-0159 and PEG 20,000 could only be tested in fresh samples of 2 and 1 dual-allergic patient(s), respectively. Results are shown as mean (connected dots) and standard error (coloured areas). Dotted line indicates cutoffs for positivity (10% for controls, 5% for other tested antigens). **B**, wb-BAT responses to BNT162b2 in dual-allergic patients (n=3) at different timepoints: before first dose (BFD, open circles), after first dose (AFD, brown circles), before second dose (BSD, open squares), 7 weeks after second dose (7wASD, closed squares), 7 months after second dose (7mASD, blue triangles). **C**, basophil response parameters (reactivity, area under curve (AUC), basophil allergen threshold sensitivity (CD sens)) in DA patients after stimulation with BNT162b2 at different timepoints (BFD, AFD, BSD, 7wASD). Dots represent values for individual patients with mean (coloured bars) and standard error bars (error bars). Horizontal bars indicate Wilcoxon matched pairs signed-rank test; ns, not significant ($p > 0.05$).

**Suggested figure width:** 2 columns

748 **FIG 4.** Transfer of IgE-dependent reactivity to BNT162b2 through PEG-allergic sera.
749 **A**, overview of allo-BAT responses in sera of healthy controls (n=4, HC1-4, green
750 triangles), dual-allergic (n=3, DA1-3, closed squares), previously PEG mono-allergic
751 (n=7, PEG1-7, open squares) and PS80 mono-allergic patients (n=2, PS1-2, blue
752 circles). Dots represent mean of 2 separate assays with the same serum. **B**, cd-BAT
753 responses in washed peripheral blood of BNT162b2-responder patients (n=3, PEG1,
754 DA2-DA3; DA3 was tested on 2 separate timepoints) reconstituted with unaltered or
755 heat-inactivated donor serum prior to stimulation. Horizontal bars indicate significance
756 level of Wilcoxon matched pairs signed-rank test. **C**, allo-BAT responses in dual-
757 allergic patient sera (n=3, DA1-3) after incubation of sensitized donor basophils with
758 dasatinib 0.25-1 µM or overnight preincubation of patient serum with omalizumab 10-
759 50 µg/mL. Mean % $CD63^+$ basophils is shown (broad coloured bars) with standard
760 error (error bars). Horizontal dotted lines indicates cutoff for positivity.

761 *Abbreviations:* BAT, basophil activation test; cd-BAT, complement-deprived BAT;
762 fMLP, N-formyl-leucyl-phenylalanine; C, complement; HDM, house dust mite.

763 **Suggested figure width:** 2 columns

764

765 **FIG 5.** Characteristics of PEG-specific IgE. **A**, allo-BAT responses to control stimuli
766 (anti-IgE and fMLP), house dust mite (HDM) extract, BNT162b2 (20 µg/mL) and ALC-
767 0159 (30 µg/mL) of stripped nonallergic donor basophils sensitized with serum of dual-
768 allergic patients (DA1-3). Serum was preincubated overnight with PBS, polyethylene
769 glycol (PEG 400-4000), diethylene glycol (DEG), or polysorbate 80 (PS80). Results
770 shown are from 3 independent experiments (each using serum from a DA patient, DA1-
771 3) with HDM only tested in serum of DA2. **B**, allo-BAT responses to BNT162b2 and
772 ALC-0159 in nonallergic donor basophils passively sensitized with 3 dilutions of dual-
773 allergic patient serum (DA1-3) or previously PEG mono-allergic serum (n=5 for
774 BNT162b2, PEG1-5; n=7 for ALC-0159, PEG1-7). **C**, CD63 dose-responses to various
775 ALC-0159-dilutions tested in whole-blood BAT (wb-BAT) on autologous patient
776 basophils (n=3, DA2-3, PEG1) and in allo-BAT using dual-allergic (n=3, DA1-3) and
777 mono-allergic sera (n=5, PEG1-5). Results are shown as mean (connected dots) +
778 SEM (coloured area) of all included assays. Dotted vertical line indicates critical
779 micellar concentration (CMC) of the related PEGylated lipid PEG 2000-DMSE (28.6
780 µg/mL). Hypothesized effect of different concentrations on ALC-0159 micelle formation
781 is shown above the graph. **D**, allo-BAT responses in sera of DA1-3 to BNT162b2 (n=3)
782 or HDM (n=1, DA2 only) w/wo pretreatment of BNT162b2 with CHAPS 0.5% prior to
783 basophil stimulation. Hypothesized disruptive effect of CHAPS detergent on lipid
784 nanoparticle structure is shown above the graph.

785 **Suggested figure width:** 2 columns

786

**FIG 6.** Serum PEG-specific IgE measured through an ImmunoCAP™ fluorescent-enzyme-immunoassay with ethylene glycol-free wash buffer. **A**, specific IgE towards PEG 2000 and PEG 10,000 expressed in kU/L measured in serum of healthy controls (n=6, green triangles), anaphylaxis controls (AC, n=15, inverted green triangles), Dual-allergic (n=3, black squares), previously PEG mono-allergic (n=6, open squares), PS80 mono-allergic (n=1, blue circle) subjects. **B**, specific IgE towards PEG 10,000 expressed in RU measured in serum of the same subjects (y-axis) *versus* corresponding total IgE values in kU/L (x-axis). **C**, relative decrease (% inhibition, leftward grey bars) or absolute decrease (RU, rightward red bars) of specific IgE values towards PEG 10,000 measured in serum of DA1-3 and AC13-14 after preincubation with BNT162b2 at a 10% v/v ratio *versus* PBS 10%. Table under graph contains absolute RU values for each condition.

All values reported are means of duplicate experiments, expressed in either in kilo-units per liter (kU/L) or arbitrary fluoresence response units (RU). Horizontal dotted lines indicate, from bottom to top, lower limit of reporting (LLR, 0.10 kU/L), standard threshold of positivity utilized by the manufacturer (0.35 kU/L corresponding with 106 RU) and upper limit of reporting (ULR, 100 kU/L).

*Abbreviations:* sIgE, specific IgE; ULR, upper limit of reporting; LLR, lower limit of reporting; RU, response units; DA, dual-allergic; AC, anaphylaxis control.

**Suggesed figure width:** 2 columns

**FIG 7.** Proposed endotype-phenotype model for excipient allergy and SARS-CoV-2 vaccine anaphylaxis including proposed methods for vaccination per subgroup. Premedication refers to H1-antihistamines.

[?] titer/affinity-dependence of the IgE endotype remains hypothetical.

[§] It is uncertain whether a PEG-containing vaccines would elicit a reaction in PEG mono-allergic patients (Picard *et al*.). In case of unavailibility of or hesitancy for PS80-containing vaccines, we offer graded vaccination with PEG-containing vaccines to our PEG mono-allergic patients.

* It is uncertain whether graded dosing is necessary in dual-allergic patients, yet it has been demonstrated to be safe. It is uncertain whether a PS80-containing vaccine would elicit a reaction in single-dose administration in dual-allergic patients. *Abbreviations*: ST, skin testing; HMW, high molecular weight; LMW, low molecular weight.

**Suggested figure width:** 2 columns

**Table I.** Overview of patient characteristics

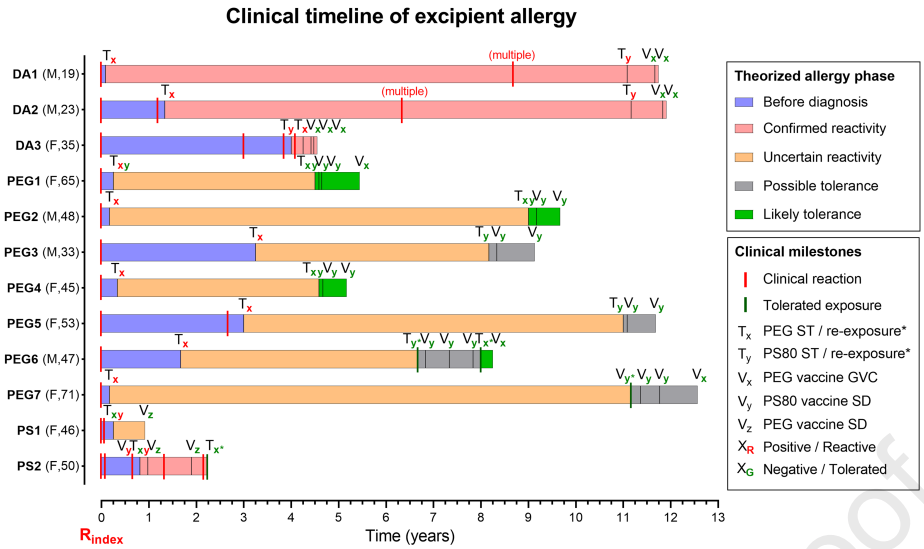
| ID | Age (at diagnosis) | Sex | Relevant history | Maintenance therapy (at diagnosis) | Culprit(s) | Reaction(s) | Skin test results | | Tryptase (µg/L) Baseline / Acute | Total IgE (kU/L) | COVID-19 vaccination history | 1) Interval last reaction 2) Interval last (+) ST * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | SPT | IDT | | | | |
| DA1 | 19 | M | - | mebeverine | 1) Iso-Betadine gel (PEG 400/4000/6000, polyvidone-iodine, topical)<br>2) Movicol (PEG 3350, oral)<br>3) cosmetic products (various PEG, topical)<br>4) Flexium gel (PEG 400, etofenamate, topical) | 1) **anaphylactic shock** (hypotension, AE, U, wheezing)<br>2) generalized U<br>3) local skin irritation | Isobetadine gel (PEG 400/4000/6000)<br>PEG 6000 (+)<br>Braunol, PEG 1500 (-) | PS80 (1/100) (+) | 3.8 / 25.2 | 3403 | BNT162b2 (2x)† | 1) 2 years, 11 months<br>2) 7 months (PS80) |
| DA2 | 23 | M | DHR to penicillin, ARC (HDM), asthma | levocetirizine, mometasone nasal spray, SABA-SAMA inhaler (on demand) | 1) calcium tablet (PEG 6000, oral)<br>2) Depo-Medrol (PEG 3350, methylprednisolone acetate, intra-articular)<br>3) sun cream (PEG 100, topical)<br>4) Iso-Betadine gel (PEG 400/4000/6000) | 1) generalized U<br>2) **anaphylactic shock** (hypotension, AE, U, wheezing)<br>3,4) local skin irritation | Depo-Medrol (PEG 3350)<br>PEG 4000<br>sun cream (PEG 100) (+)<br>Solu-Medrol, PEG 400 (-) | PS80 (1/10), PEG 1500 (1/10) (+)<br>Solu-Medrol, PEG 400 (-) | 5.3 / NA | 152 | BNT162b2 (2x)† | 1) 9 years, 10 months<br>2) 8 months (PS80) |
| DA3 | 35 | F | - | - | 1) Movicol (PEG 3350, oral)<br>2) Diprophos (PEG 4000/PS80, betamethasone dipropionate, intra-articular)<br>3) Moviprep (PEG 3350, topical§)<br>4) Cordarone (PS80, amiodarone, topical§)<br>5) Cosmetic products (various PEG, topical) | 1,2) **anaphylactic shock** (hypotension, AE, U, abdominal cramping)<br>3,4) presyncope, palpitations, generalized erythema<br>5) local skin irritation, generalized erythema | Movicol (PEG 3350) (+) | PS80 (1/100)§ (+) | 4.3 / NA | 296 | BNT162b2 (3x)† | 1) 5 months<br>2) 1 month (PEG 3350) |
| PEG1 | 65 | F | OAS, food allergy (hazelnut) | aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin | Depo-Medrol (PEG 3350, intra-articular) | **anaphylactic shock** (hypotension, diarrhoea, nausea) | Depo-Medrol (PEG 3350)<br>PEG 4000, PEG 6000 (+);<br>lidocaine, chlorhexidine, Solu-Medrol, PEG 400, PEG 1500 (-), MedrolΔ (-) | PS80 (1/10), lidocaine, chlorhexidine, Solu-Medrol (-) | 9.8ξ / 32.5 | 13.8 | Janssen (2x)<br>BNT162b2 (1x)† | 1) 4 years, 2 months<br>2) 4 years |
| PEG2 | 48 | M | ARC (grass pollen) | asaflow, rosuvastatin | Diprophos (PEG 4000/PS80, intra-articular) | **anaphylactic shock** (hypotension, generalized pruritus) | PEG 4000 (+), Solu-Medrol, Solu-Cortef, Aacidexam, Volon, PEG 400 (-) | Depo-Medrol (PEG 3350), Diprophos (+), PS80 (1/10) (-) | 4.8 / 23.8 | 22.5 | Janssen (2x) | 1) 8 years, 6 months<br>2) 8 years, 4 months |
| PEG3 | 33 | M | - | - | Depo-Medrol (PEG 3350, intra-articular) | generalized U, abdominal cramping, vomiting | Depo-Medrol (PEG 3350)§, PEG 4000§ (+), MedrolΔ (-) | PS80 (1/10) (-) | 6.3 / NA | 74 | Janssen (1x)<br>Nuvaxovid (1x) | 1) 7 years, 1 month<br>2) 6 years |

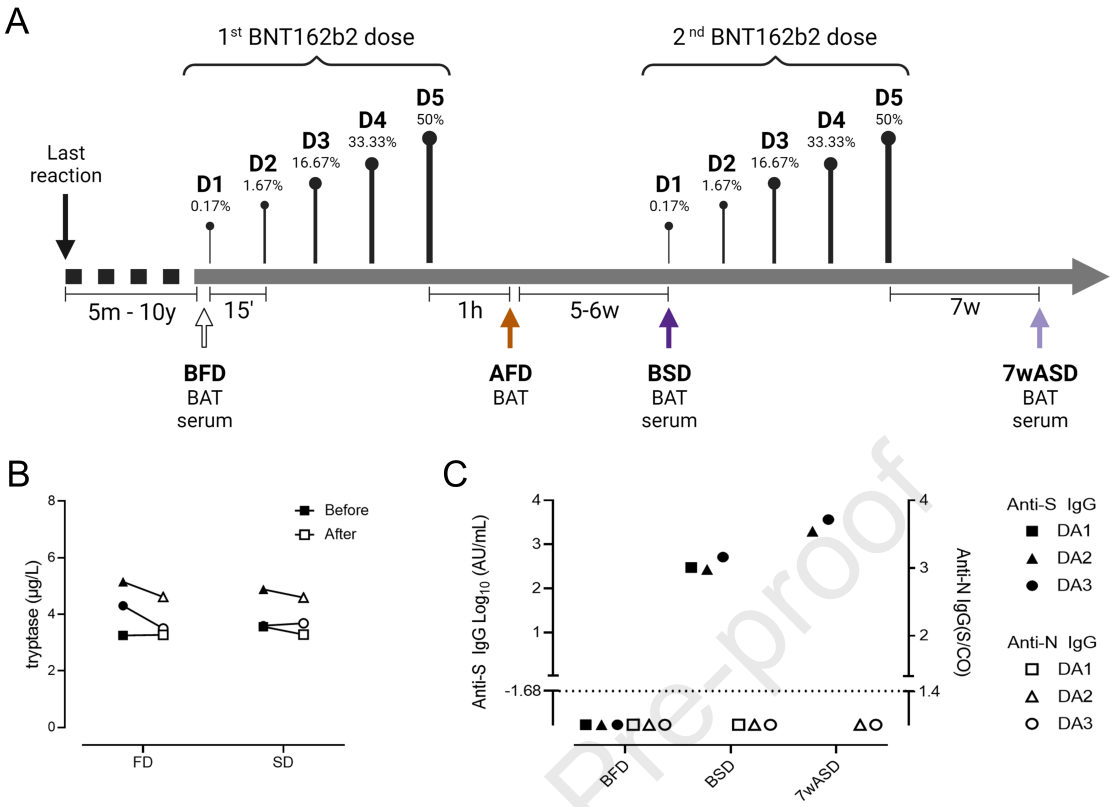
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PEG4 | 45 | F | - | - | Depo-Medrol (PEG 3350, intra-articular) | generalized U, vomiting | Chlorhexidine, latex, bupivacaine, Solu-Medrol, Celestone (-), Medrol$^{\Delta}$ (-) | PS80 (-), Depo-Medrol (PEG 3350) (-)$^{\$}$ | 4.7 / NA | 169 | Janssen (2x) | 1) 4 years, 11 months<br>2) 4 years, 7 months |
| PEG5 | 53 | F | - | - | 1) Moviprep (PEG 3350, oral)<br>2) Depo-Medrol (PEG 3350, intra-articular) | 1,2) generalized U, AE, wheezing | - | PEG 4000$^{\$}$<br>Depo-Medrol (PEG 3350)$^{\$}$ (+), PS80, Solu-Medrol, Solu-Cortef, Celestone, PEG 400 (-) | 2.8 / NA | 1778 | Janssen (2x) | 1) 10 years, 6 months<br>2) 7 years, 5 months |
| PEG6 | 47 | M | - | bisoprolol | Depo-Medrol (PEG 3350, intra-articular) | generalized U and erythema, atrial fibrillation, nausea | PEG 4000, PEG 6000 (+), Solu-Medrol, bupivacaine (-) | Depo-Medrol (PEG 3350), PEG 4000 (1/1000) (+), PEG 400, PEG 1500 (-), PS80$^{\Delta}$ (-) | 4.4 / NA | 29 | Janssen (2x)<br>Nuvoxavod (1x)<br>BNT162b2 (1x)$^{\dagger}$ | 1) 11 years, 9 months<br>2) 8 years, 6 months |
| PEG7 | 71 | F | - | rosuvastatin, dosulepin, montelukast | 1) Depo-Medrol (PEG 3350, intra-articular)<br>2) Bactroban (PEG 400/3350, mupirocine, topical) > Lotaradine Mylan (PEG 400/6000, oral) | 1) unknown systemic reaction<br>2) generalized U | mupirocine, loratadine (-) | PEG 400, PEG 4000 (1/10) (+), Depo-Medrol (PEG 3350), Solu-Medrol (-), PS80$^{\Delta}$ (-) | 7.3 / NA | 70 | Vaxzevria (2x)<br>Janssen (1x)<br>BNT162b2 (1x)$^{\dagger}$ | 1) 11 years, 1 month<br>2) 10 years, 11 months |
| PS1 | 46 | F | breast cancer | - | 1) Taxotere (PS80, docetaxel) 2) Pelmeg (PS20, PEG 20,000, rhGM-CSF) | 1) generalized U, nausea<br>2) **anaphylactic shock** (hypotension, generalized erythema, diarrhoea) | Taxotere (PS80) (+) | PS80 (1/100) (+)<br>PEG 400-20.000 (-), PEG 2000 $^{\Delta}$(-) | 2.4 / 4.7 | 1955 | BNT162b2 (3x) | 1) 8 months<br>2) 2 months |
| PS2 | 50 | F | ALL with bone marrow transplant, myasthenia gravis, rheumatoid arthritis | L-thyroxine, pyridostigmine, SAMA-SABA inhaler (on demand) | 1) Mabthera (PS80, rituximab)<br>2) Vaxzevria (PS80) | 1,2) **anaphylactic shock** (hypotension, AE, U, wheezing) | PS80 (1/1) (+), PEG 20,000 (1/1) (-) | PEG 3350 (1/10) (-) | 4.8 / 5.9 | 22 | Vaxzevria (1x)$^{\$}$<br>BNT162b2 (2x) | 1) 6 months<br>2) 10 months |

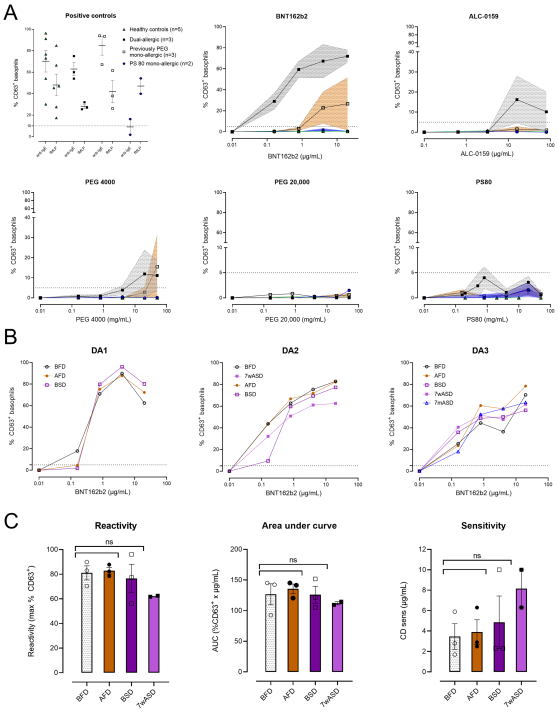
Profession of the different patients included (in alphabetical order) administrative clerk, factory worker, garbage collector, nurse, physiotherapist, policeman, professional driver, teacher.

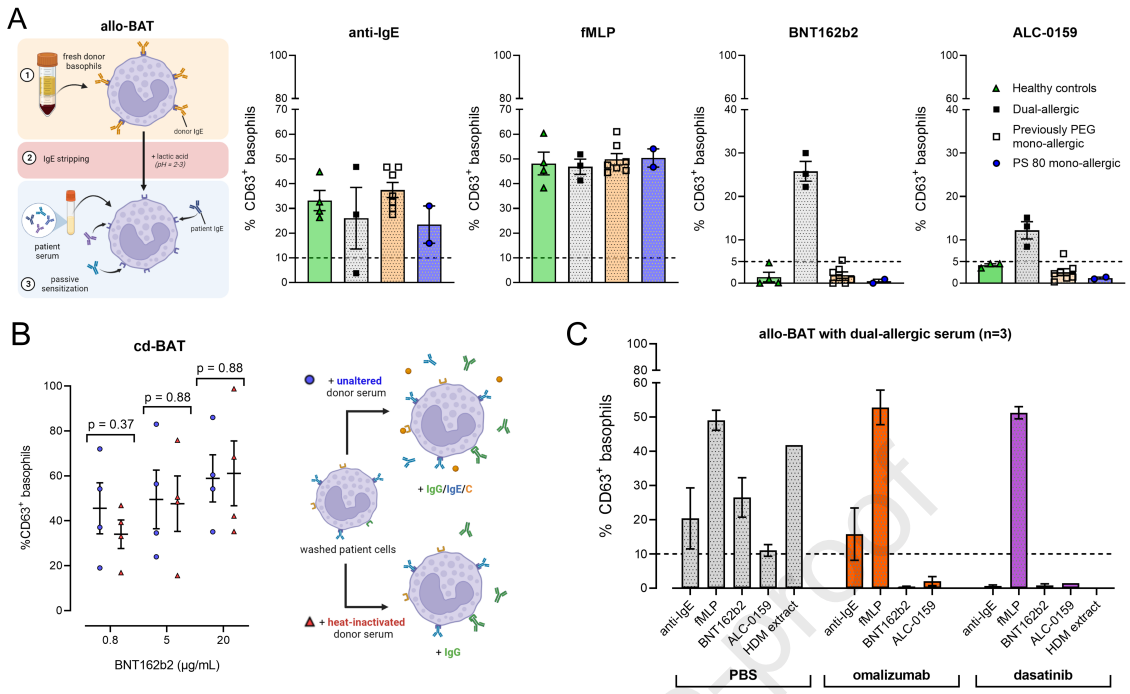
* Interval to first vaccine dose for dual-allergic patients or date of serum sampling for mono-allergic patients. † Vaccine administration through graded vaccine challenge protocol; § Allergic reaction occurred after skin contact and/or inhalation while patient was handling medication; $ Systemic reaction during skin testing or vaccination. δ Allele-specific qPCR on peripheral blood did not detect a somatic c-KIT D816V mutation. Δ Documented tolerated exposition, either through oral provocation testing or through single-dose administration of a non-SARS-CoV-2 PS80-containing vaccine. Extensive chronological information on each clinical case is provided in the online repository under clinical vignettes.

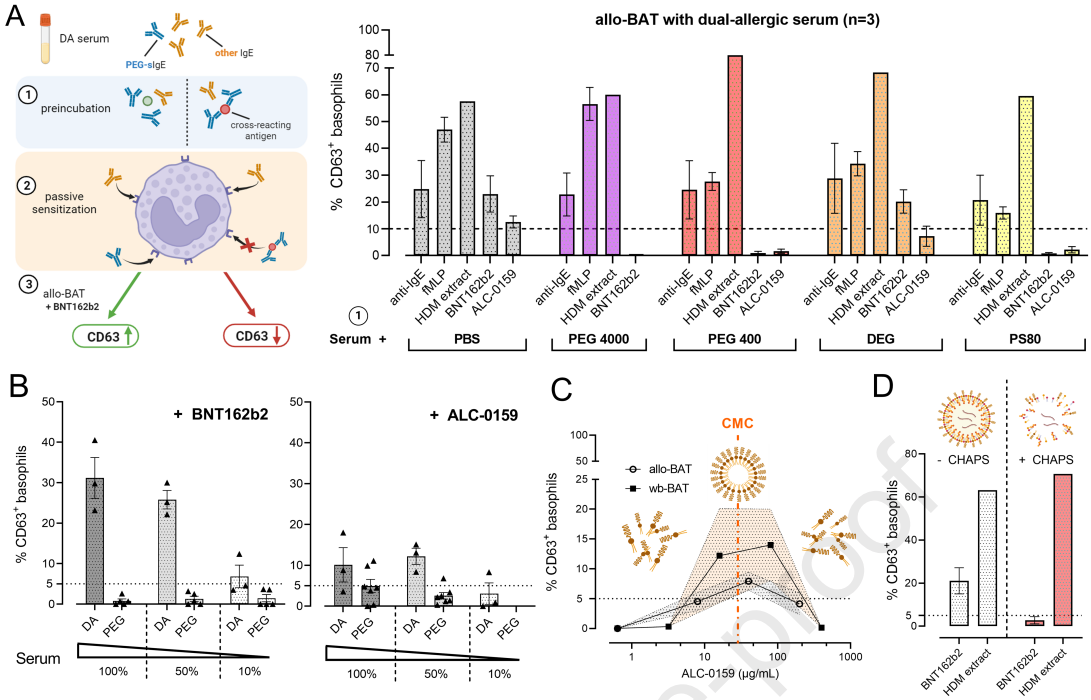
*Abbreviations:* SPT, skin prick test; IDT, intradermal test; OPT, oral provocation test; ST, skin test; AE, angioedema; U, urticaria; DHR, delayed hypersensitivity reaction; ARC, allergic rhino-conjunctivitis; OAS, oral allergy syndrome; SABA-SAMA, short-acting beta-2 agonist / muscarinic antagonist; HDM, house dust mite; ALL, acute lymphocytic leukemia; NA, not available.

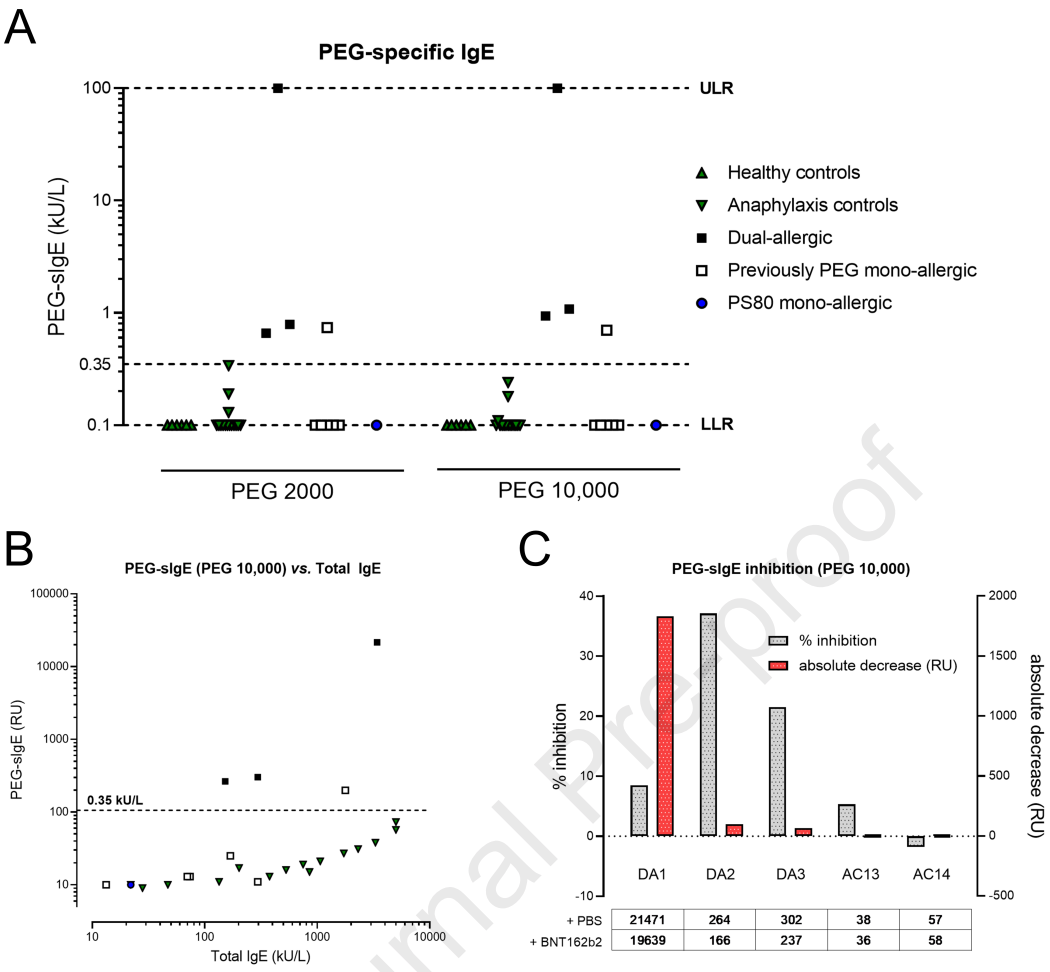


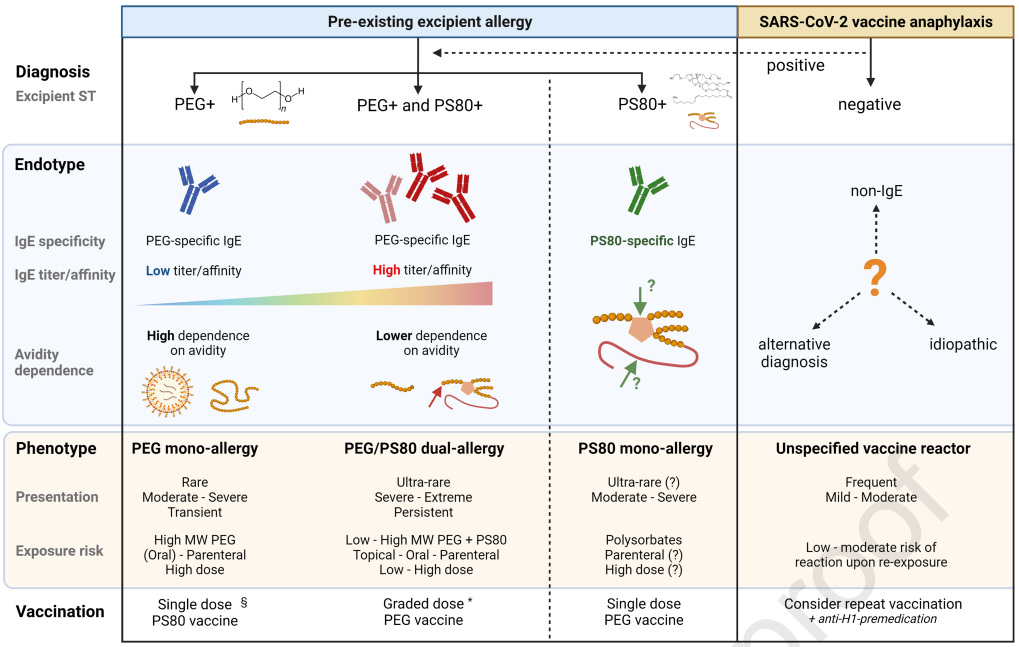


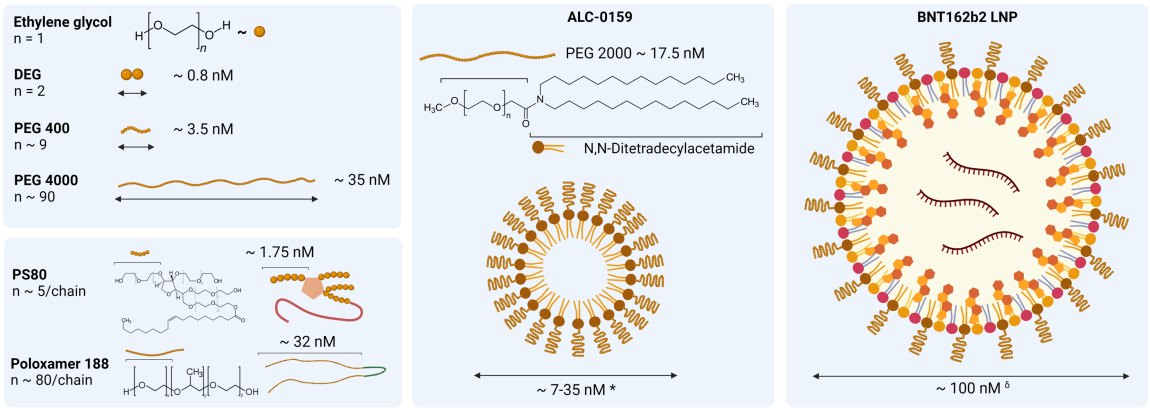


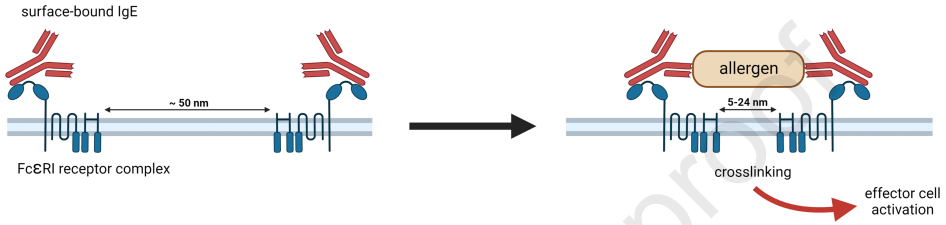




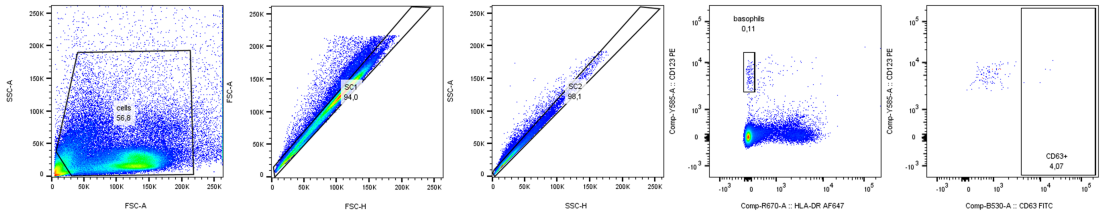




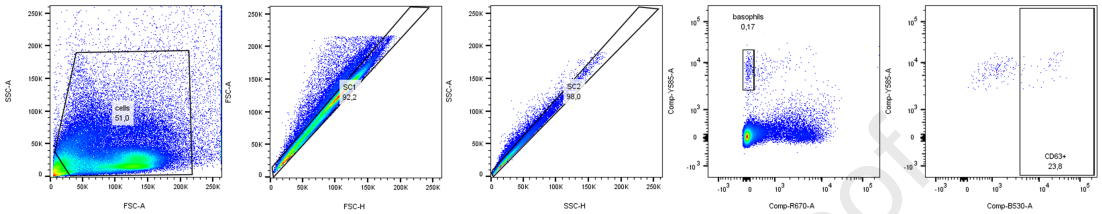


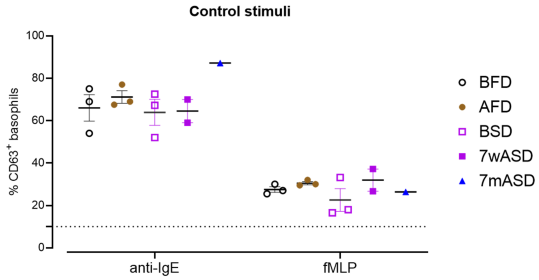



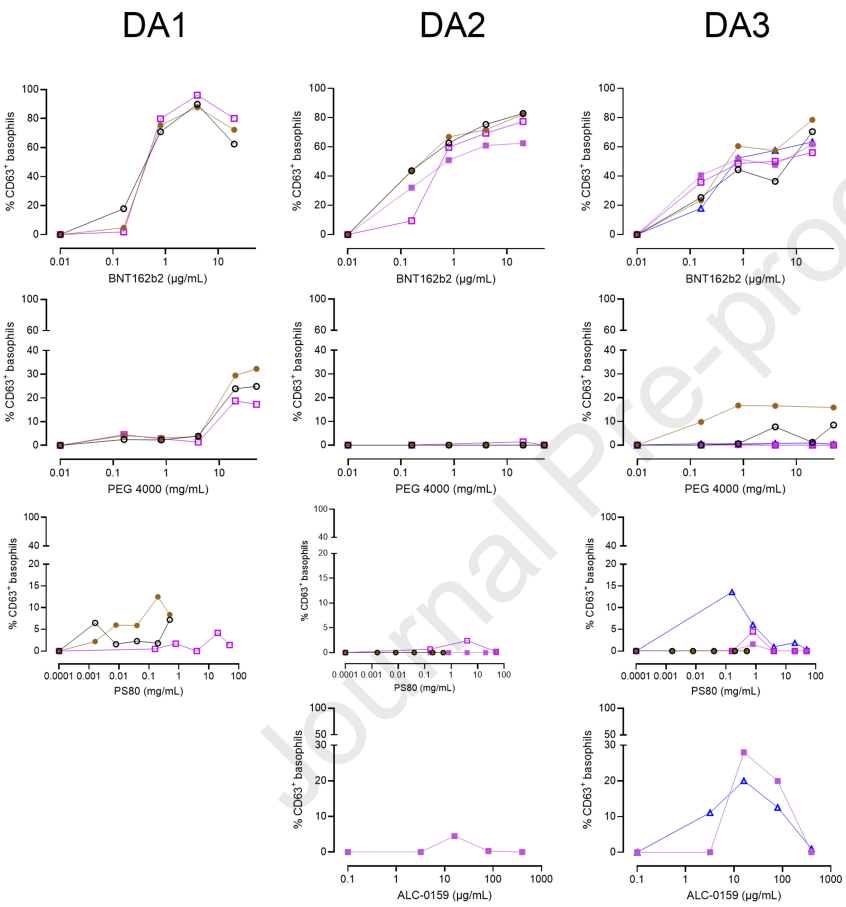
BSB (+ IL-3)

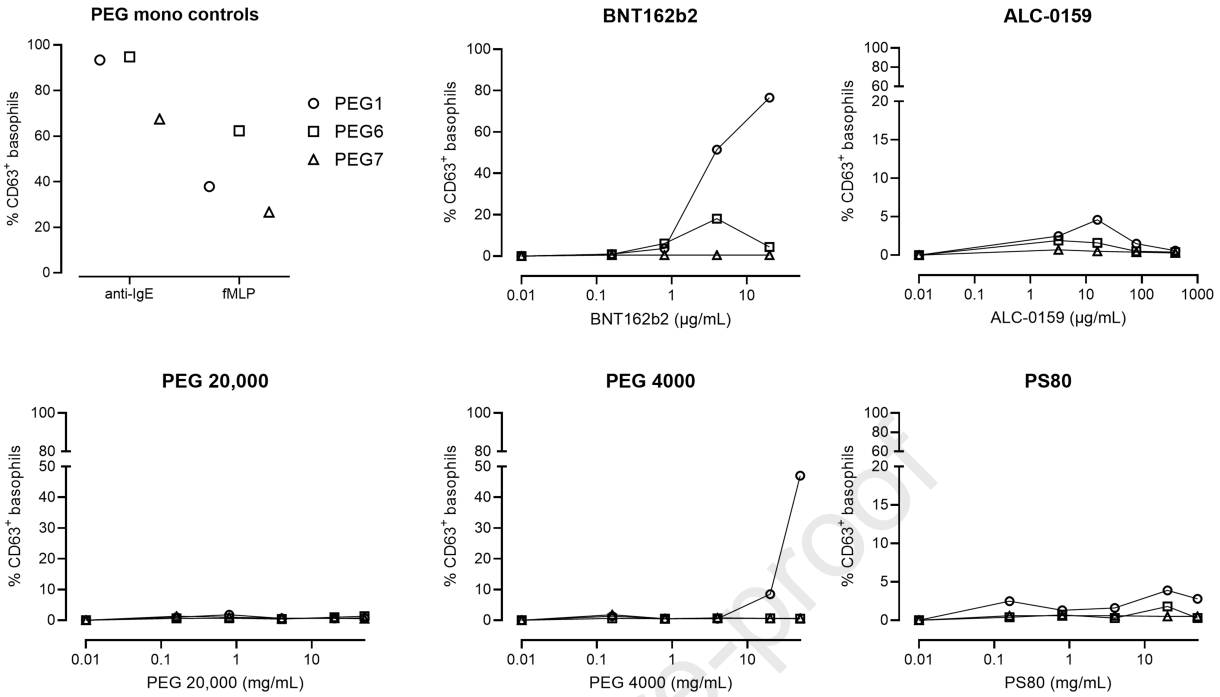



BNT162b2 20 µg/mL

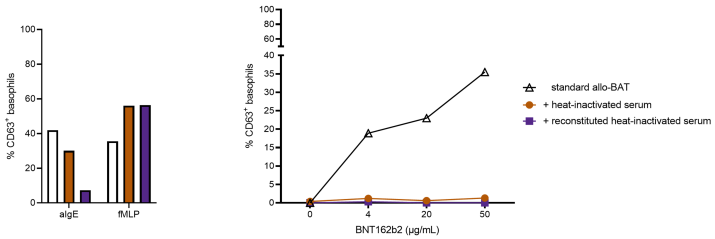




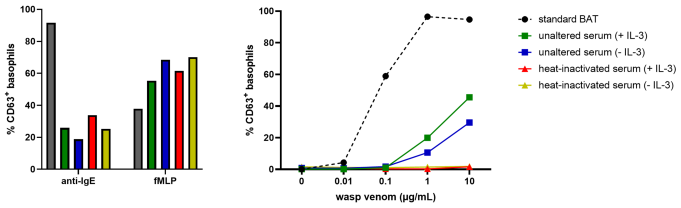



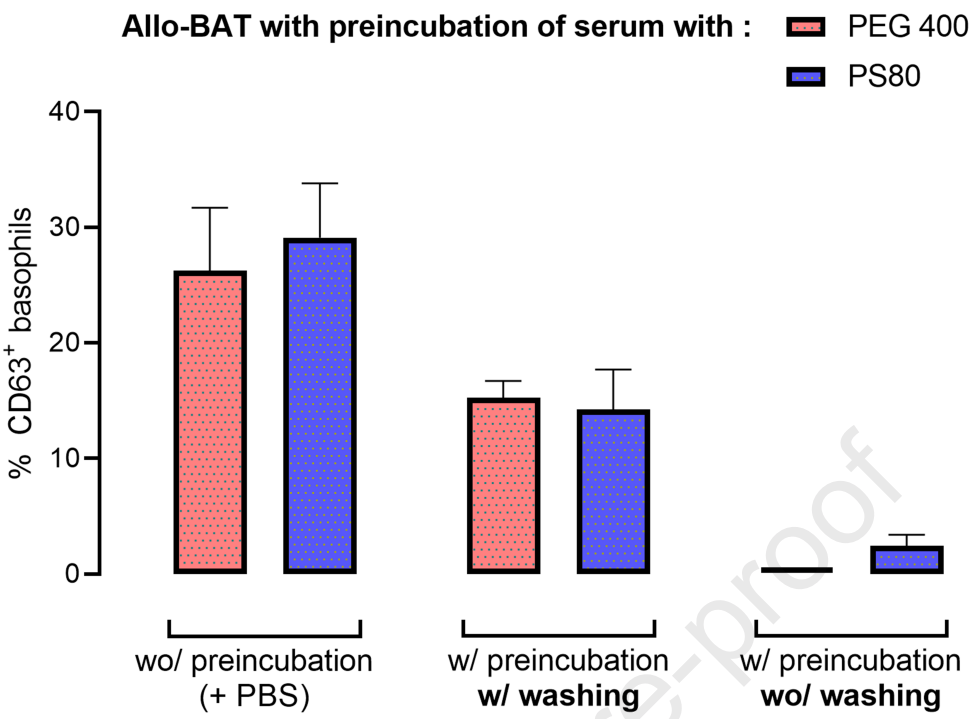


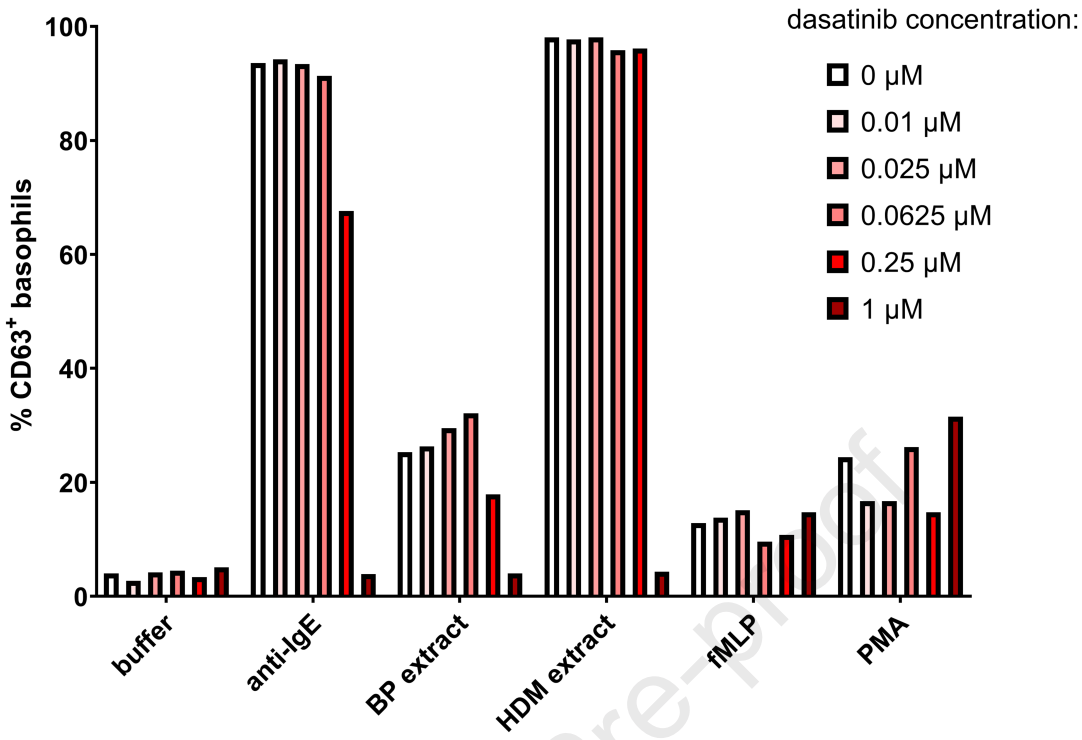
**A allo-BAT experiment in a dual-allergic patient (DA3)**

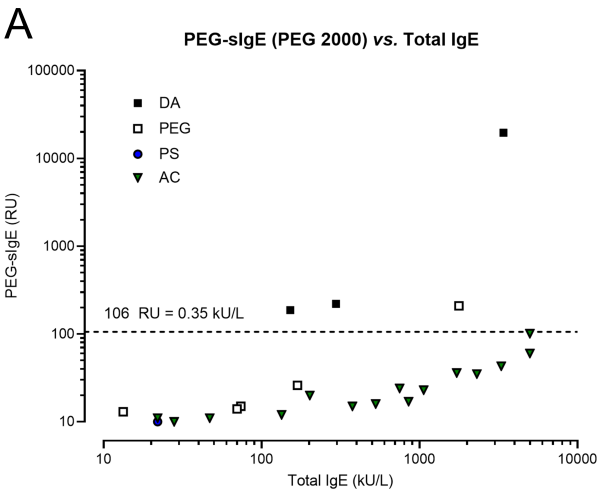


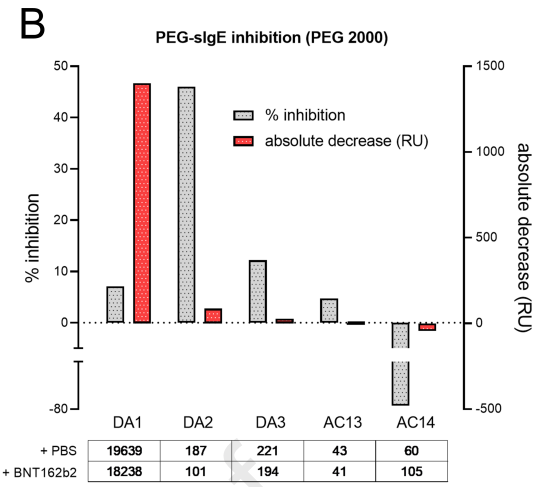

**B allo-BAT *vs.* wb-BAT in wasp venom allergic control**









**Online repository**

Supplementary methods

- Study protocol and ethics approval (S60734)
- Skin test procedure
- Graded vaccine challenge protocol
- Blood sampling procedure
- Serological analyses
- Whole blood basophil activation test (wb-BAT) protocol
- Complement-deprived basophil activation test (cd-BAT) protocol
- Allo-basophil activation test (allo-BAT) protocol
- Inhibition of IgE-dependent basophil activation

Clinical vignettes

Tables

- **Table E1**. Graded vaccination challenge (GVC) protocol with BNT162b2.
- **Table E2.** Timing of sample collection for *in vitro* assays.
- **Table E3.** Biochemical characteristics of excipient allergic subjects
- **Table E4.** Characteristics of anaphylaxis controls
- **Table E5.** Characteristics of healthy controls.
- **Table E6**. Reagents for basophil activation testing (BAT)
- **Table E7.** Basophil reactivity to tested antigens in whole blood of patients and controls.
- **Table E8.** Donor basophil reactivity to tested antigens in allo-BAT after passive sensitization with serum of patients and controls.

- **Table E9.** Total and PEG-specific IgE levels directly measured in serum of patients and controls (ImmunoCAP™)

Figures

- **Fig E1**. Schematic size comparison of ethylene glycol-based excipients and size requirements for IgE cross-linking.
- **Fig E2**. Flowcytometric gating strategy for BAT experiments
- **Fig E3**. Whole blood basophil activation test results in DA patients at different timepoints.
- **Fig E4**. Whole blood basophil activation test results in previously PEG mono-allergic patients.
- **Fig E5**. Preliminary allo-BAT experiments.
- **Fig E6**. Allo-BAT inhibition on DA sera with PEG 400 and PS80 with/without washing prior to stimulation with BNT162b2
- **Fig E7**. Effect of dasatinib pretreatment on whole blood BAT responses to various IgE-dependent and IgE-independent stimuli.
- **Fig E8.** Serum PEG-specific IgE measured through an ImmunoCAP™ fluorescent-enzyme-immunoassay with ethylene glycol-free wash buffer.

References

## Supplementary methods

### Study protocol

Excipient allergic patients and anaphylaxis controls (AC) were included in the prospective study titled 'extensive *ex vivo* investigation into causes of anaphylaxis' approved by the Ethics Committee Research UZ/KULeuven (study number S60734). The goal of this study is the discovery and validation of novel culprit allergens in anaphylaxis. Inclusion criteria encompass all adult patients with a history of anaphylaxis of undetermined (primary study population) or determined causes (disease controls) seen on at least one occasion for diagnostic workup at the outpatient allergy clinic of University Hospitals Leuven, a large tertiary referral center in Leuven, Belgium (1949 inpatient beds). The study provides a practical and ethical framework for prospective collection of fresh blood samples for *ex vivo* analyses (basophil activation testing) as well as long-term biobanking of serum samples for additional *in vitro* analyses at later timepoints (mass spectrometry, immunoblotting, ELISA/FEIA, …). All patients were required to provide written informed consent prior to sampling.

### Skin test procedure

Patients were diagnosed over a 12-year period, between 2009 and 2021. Skin testing (ST) procedures evolved over time, in line with the prevailing literature. ST for polysorbate 80 (PS80) was not systematically performed in all patients throughout this period and was completed at a later timepoint prior to SARS-CoV-2 vaccination, if not performed at initial diagnosis and deemed clinically necessary. ST was performed according to a previously published protocol.[E1] In brief: the current ST protocol includes sequential testing with PS80 (Tween 80, 1 mg/mL in sterile water

for injection; *Fagron, Belgium*) skin prick test (SPT) undiluted followed by intradermal testing (IDT) up to 0.1 mg/mL (further dilutions in NaCl 0.9%). Polyethylene glycol (PEG) is evaluated using sequential SPT with undiluted PEG 400 (*Fagron, Belgium;* no concentration provided by the manufacturer), PEG 3350 (Depo-Medrol 40 mg/mL methylprednisolone acetate; and PEG 3350 29 mg/mL), PEG 3350 (Movicol, 100 mg/mL), PEG 4000 (macrogol, 100 mg/mL In sterile water for injection; *Fagron, Belgium*), PEG 20,000 (Flagyl, metronidazole 500 mg/tablet; and PEG 20,000 1.4 mg/700 mg [0.2%] tablet). PEG dilutions for SPT (1/10–1/1000 in NaCl 0.9%) are used only in case of a severe index reaction or high index of suspicion for genuine IgE-mediated PEG allergy. IDT with PEG are currently only performed with Depo-Medrol (PEG 3350 up to 2.9 mg/mL, 1/10 dilution) when probability of PEG allergy is deemed to be low or in case of confirmed PS80 allergy and necessity to demonstrate tolerance to PEG (as in the context prior to Pfizer/BioNTech or Moderna SARS-CoV-2 vaccination). All ST are performed with 30-minute intervals and in a monitored setting (with intravenous access in patients with a history of anaphylaxis or in those who receive IDT with Depo-Medrol). The SPT with PEG 20,000 was added to the ST protocol in May 2021. Positive (histamine 10 mg/mL) and negative (0.9% saline) SPT controls are always performed at the beginning of the ST protocol. For IDT, a volume of 0.05 mL is used per injection.

Graded vaccine challenge protocol

Graded vaccine challenges with BNT162b2 (Comirnaty®, Pfizer-BioNTech) were performed in all SARS-CoV-2 vaccine naive dual PEG and PS80 allergic patients and selected previously PEG mono-allergic patients according to a 5-step protocol with 15-minute intervals (starting at 0.05 mL of a 1/100 dilution or 0.17%, over 0.05 ml of a 1/10 dilution or 1.67%, to 0.05 mL or 16.67%, 0.1 mL or 33.33% and finally

0.15 ml of undiluted or 50% of the standard adult dose of the BNT162b2 vaccine) resulting in a cumulative dose of 0.31 mL or 101.84%, as previously reported by Huyhn *et al*.[E2] This protocol was adapted from the standard GVC protocol for vaccines proposed by the AAAAI/ACAAI joint task force on adverse reactions to vaccines in the 2012 practice parameter update.[E3] Vaccine dilutions were prepared in the hospital pharmacy using sterile water for injection (USP). Vaccination was performed in-hospital under direct allergist supervision, unilaterally in the upper arm, without premedication and tolerability was assessed on-site. Total time spent in-hospital for the GVC was around 2 hours, including preparation and 30-minute observation after the final dose step. All dual allergic patients received two BNT162b2 vaccines through this protocol with a 5-6 week interval between both procedures. See also **Table E1** for more details on the GVC protocol.

Blood sampling procedure

Blood samples for basophil activation testing (BAT) and serum were collected in lithium heparin and serum separator tubes (BD Vacutainer), respectively. In dual allergic patients, samples were obtained concurrently, immediately prior to the first dose-step and 1 hour after administration of the final dose-step (see **figure 1A**) at both vaccine challenges. Additional follow-up samples were obtained through an outpatient visit 7 weeks after the second dose in DA2 and DA3 and 7 months after the second dose in DA3. Standard whole blood BAT analyses were performed within 1 hour after sampling. Serum samples were processed within 12 hours after sampling and stored at -80°C prior to use in experiments. For mono-allergic patients, samples were obtained at various timepoints in relation to initial diagnosis and vaccination. Timing of blood and serum sampling for wb-BAT and serum (for allo-

BAT and PEG-specific IgE measurement), respectively, relative to the index reaction is given for each patient in **Table E2.**

Serological analyses

*Anti-SARS-CoV-2 IgG* were measured in serum samples of dual allergic patients obtained prior to administration of both vaccines doses. Anti-N (nucleocapsid) IgG levels were determined through a semi-quantitative chemiluminescent microparticle immunoassay (CMIA) using a signal/cut-off value ≥ 1.40 for positivity. Anti-S (spike) IgG levels were measured using a quantitative CMIA assay using the 50 AU/mL cut-off for positivity as per the manufacturer's instructions. Analyses were performed on an Architect i2000SR analyzer (Abbott, Lake Forest IL, USA).

*Serum tryptase and total IgE* were measured using the ImmunoCAP fluorescent enzyme immunoassay on a Phadia 1000 analyzer (Phadia ThermoFisher, Upssala, Sweden). in the clinical laboratory of University Hospitals Leuven.

*Serum PEG-specific IgE* were measured using a research use only (RUO) ImmunoCAP assay for PEG 2000 (U1337) and PEG 10,000 (U1348) provided through the ImmunoCAP PEG assay test service at the Phadia laboratories (Uppsala, Sweden). All assays were performed in duplicate on an ImmunoCAP 250 analyzer using a specially prepared washing solution where the standard additive was exchanged with an ethylene glycol-free alternative consisting of a 98% solution of 1-O-n-Octyl-β-D-glucopyranoside in water (ThermoScientific Acros, product code 10541794). Hu-6.3-IgE (Academia Sinica) was used as positive control for this assay. For ImmunoCAP inhibition assays, 90 µL serum was preincubated with 10 µL of undiluted BNT162b2 or PBS prior to analysis.

Whole blood basophil activation test (wb-BAT) protocol

One hundred fifty microliter aliquots of fresh lithium heparin whole blood were incubated for 25 minutes at 37°C with 30 µL of various stimuli dissolved in cBSB including negative control (cBSB without additives) and positive controls (aIgE, fMLP). An overview of all reagents used, including applied concentrations, is given in **Table E1.** Reactions were stopped by incubation on ice for 5 minutes followed by staining with 4 µL of pre-titrated fluorochrome-conjugated antibody mix (anti-CD123 PE, anti-HLA-DR Alexa Fluor 647 and anti-CD63 FITC) for 25 minutes at 4°C. Following staining, red blood cells in stained samples were lysed through addition of 2 mL FACS lysis buffer, followed by washing in PBS and fixation in 1% paraformaldehyde. Samples were acquired on an LSRFortessa flowcytometer equipped with FACSDiva software and analyzed using FlowJo v10.8.1 (Beckton Dickinson, San Jose CA, USA). Basophils were gated as $CD123^{+}$/$HLA\text{-}DR^{-}$ cells with at least 150 basophils acquired per sample. A cut-off of 5% $CD63^{+}$ basophils, after subtraction of the percentage $CD63^{+}$ basophils in the negative control sample, was used to determine positivity in accordance with expert consensus.[E4] An example of the gating strategy is shown in **Fig E2**. BAT outcome parameters analysed included basophil *reactivity* (maximum % $CD63^{+}$ basophils across all concentrations of a given stimulus), *sensitivity* expressed as CDsens (inverse of the concentration of a given stimulus required to elicit half-maximal basophil activation) and *area under the dose-response curve* (AUC).[E4]

Complement-deprived basophil activation (cd-BAT) test protocol

To assess the impact of heat-labile serum components on basophil reactivity to various stimuli, 3 mL fresh whole blood was centrifuged at 1500 g for 5 minutes and

the plasma layer was carefully removed, leaving formed elements including autologous patient basophils sensitized with autologous IgE. These cells were washed twice in RPMI-1640 supplemented with 1% HSA and 10 IU/mL heparin to remove all remaining plasma. Washed cells were split in two equal 750 µL aliquots and reconstituted with 750 µL of serum from a nonallergic donor. Donor serum was either unaltered or heat inactivated prior to reconstitution. Heat inactivation of serum samples was performed by heating for 30 minutes at 56°C in a hot water bath with gentle inversion at 10 minute intervals, eliminating heat-labile components including complement and leading to denaturing of the receptor-binding Fc domain of free IgE, leaving heat-stable immunoglobulins such as IgM and IgG intact.[E5-6] Further sample processing and analysis was identical to the standard wb-BAT protocol.

Allo-basophil activation testing protocol (allo-BAT)

Allo-BAT experiments used basophils from a nonallergic donor with confirmed IgE-responder status on wb-BAT after stripping of autologous donor IgE and passive sensitization with allogenic patient IgE. Stripping and sensitization were performed according to a protocol reported previously by Yasui *et al*.[E7] In brief: fresh lithium-heparin blood was obtained from a single non-allergic donor with consistent CD63 expression >70% in response to aIgE on standard wb-BAT. This donor had demonstrated clinical tolerability to multiple doses of a PEG-containing SARS-CoV-2 vaccine and had no prior SARS-CoV-2 infection. Peripheral blood mononuclear cells (PBMC) were isolated from donor blood using density gradient centrifugation over a 1.077 g/L density medium (Lymphoprep, StemCell Technologies, Vancouver, Canada) and remaining medium was removed through successive washing steps. The resulting cell suspension contained mononuclear cells and low-density granulocytes. Surface-bound autologous IgE was stripped from PBMC in a 13.4 mM

lactic acid buffer (5 x $10^6$ PBMC / mL) during 5 minutes on ice. Stripping was stopped through addition of an equal volume of neutralization buffer (RPMI-1640 with 0.5% HSA and 12 mM Tris-HCl at pH 8.0) followed by 5 minutes centrifugation at 1200 g at 4°C. After an additional washing step, stripped donor basophils were passively sensitized with heterologous IgE through incubation in patient serum at 37°C for 60 minutes in polypropylene tubes. PBMC maintained > 80% viability after successive isolation, stripping and sensitization steps as assessed through trypan blue staining. Sensitized basophils suspended in patient serum were transferred to polystyrene FACS tubes containing 30 µL stimulus dissolved in stimulation buffer in 150 µL aliquots containing 2.5 – 5 x $10^5$ PBMC. All subsequent steps were identical to the whole blood BAT protocol excluding the RBC lysing step. For allo-BAT inhibition experiments, patient serum was preincubated with different inhibitors in a 1:1 v/v ratio at 4°C overnight, prior to passive sensitization. For allo-BAT inhibition experiments, serum for baseline response determination was preincubated simultaneously with a 1:1 v/v ratio of PBS to adjust for dilution.

In a supplementary experiment, the impact of the presence or absence of inhibitor in the reaction medium during stimulation with BNT162b2 was assessed through inclusion of a condition with washing. Here, donor basophils, suspended in patient serum with inhibitor, were washed twice in RPMI after sensitization to remove all remaining serum and subsequently reconstituted in serum of the basophil donor prior to addition of the stimulus (**Fig E6**).

<u>Inhibition of IgE-dependent basophil activation</u>

To inhibit FcεRI-dependent basophil activation, samples were preincubated with dasatinib 1 µM (Toronto Research Chemicals, Toronto, Canada) for 15 minutes at

212 37°C prior to stimulation according to a protocol described by Kneidinger *et al.*[E8]
213 Dasatinib is a multikinase inhibitor that inhibits downstream signalling through the
214 high-affinity IgE receptor through inhibition of Bruton's Tyrosine Kinase (BTK). IgE-
215 specificity of dasatinib's inhibitory effect was confirmed in a pilot experiment using
216 fresh blood of HC4 (**Fig E7**).

217 In a second, separate experiment, serum samples were preincubated with either
218 diluent (PBS) or omalizumab (ProteoGenix, France), a humanized monoclonal IgG
219 which selectively binds to the Fc domain of IgE preventing its binding to the high-
220 affinity IgE receptor. Serum was incubated overnight with omalizumab at a final
221 concentration of 10-50 µg/mL, in order to reach a stoichiometric excess omalizumab
222 to IgE ratio. Treated serum samples were subsequently used to passively sensitize
223 stripped donor basophils prior to stimulation with BNT16b2 and ALC-0159.

224

## Clinical vignettes

**DA 1** – A 19-year-old male without history of allergic disorders was referred to the outpatient allergy clinic 30 days after presentation at the emergency department with severe anaphylaxis (hypotension, wheezing, urticarial rash and facial angioedema) with tryptase elevation (25.2 µg/L *versus* 3.8 µg/L at baseline) treated with epinephrin. The reaction occurred within 10 minutes after application of a PEG 400/4000/6000-containing polyvidone-iodine gel (Iso-betadine) under occlusion on an open wound on the right elbow. Skin prick tests were positive for Iso-betadine gel and PEG 6000 but negative for PEG 1500 and Braunol (polyvidone-iodine 7.5% solution containing PEG-9 lauryl alcohol). He was diagnosed with PEG allergy and advised to avoid all PEG-containing products. The patient was recalled 11 years later for additional skin testing prior to COVID-19 vaccination and reported multiple reactions since the initial diagnosis including a systemic reaction (angioedema) after accidental oral intake of a PEG 3350-containing laxative (Movicol®) treated with epinephrin as well as local skin irritation and itching after application of multiple PEG-containing topical agents including a PEG 400-containing NSAID gel (Flexium), shower gel and shaving cream. Intradermal skin test with PS80 (1/100) was positive at this time and the patient was excluded from COVID-19 vaccination due to dual sensitization to PEG and PS80. He tolerated both doses of the PEG-containing BNT162b2 vaccine using a graded challenge protocol 7 months later.

**DA 2 –** A 23-year-old male with a history of allergic rhino-conjunctivitis, asthma with underlying skin-test proven house dust mite and grass pollen allergy and delayed-type hypersensitivity to penicillin was referred to the outpatient allergy clinic 2 months after a severe anaphylactic reaction (syncope, generalized erythema, urticaria, wheezing, facial angioedema) occurring within 1 minute after injection of a PEG

3350-containing methylprednisolone acetate solution (Depo-Medrol) in the groin, treated with epinephrine. The patient also reported a generalized urticarial reaction after oral administration of a PEG 6000-containing calcium tablet (Calcium Sandoz) 1 year prior to the index event as well as local skin irritation upon application of a PEG 100-containing sun cream. Skin prick tests with Depo-Medrol as well as PEG 4000 and an intradermal test with PEG 1500 were positive whereas skin prick and intradermal tests with methylprednisolone sodium-succinate (Solu-Medrol) and PEG 400 were negative. The patient was diagnosed with PEG allergy and advised to avoid all PEG-containing products. The patient was recalled 10 years later for additional skin testing prior to COVID-19 vaccination and reported multiple occurrences of immediate rash upon application of Iso-Betadine gel (PEG 400/4000/6000) since initial diagnosis. Intradermal skin test with PS80 (1/10) was positive at this time and the patient was excluded from COVID-19 vaccination due to dual sensitization to PEG and PS80. He tolerated both doses of the PEG-containing BNT162b2 vaccine using a graded challenge protocol 8 months later.

**DA 3 –** A 35-year-old female without history of allergic disorders was referred to the outpatient allergy clinic for work-up prior to COVID-19 vaccination due to a history of multiple reactions to PEG-containing products in the past: 1) 4 years prior, 20 minutes after intake of an oral PEG 4000-containing laxative (Movicol®), she experienced onset of palpitations, pruritus, angioedema of face and hands and generalized urticaria culminating in loss of consciousness. 2) 1 year prior, she received an intramuscular injection with a PEG 4000/PS80-containing betamethasone dipropionate solution (Diprophos) resulting in immediate onset of palpitations, abdominal cramping, pruritus, cough, angioedema of hands and feet and loss of consciousness; 3) 3 months prior, she experienced immediate onset of

palpitations and pruritus at work during preparation of a PEG 4000-containing bowel prep for a patient, which she treated with an oral H1-antihistamine; 4) she reported multiple episodes of generalised erythema after exposure to PEG-containing shower cream and tooth paste products. Intradermal skin testing with PS80 (1/100) at first consultation was positive and accompanied by a mild systemic reaction similar to previous reactions (generalized erythema, palpitations, pruritus). Due to the systemic reaction, skin testing with PEG was postponed to a subsequent consultation 2 months later during which she also reported having had a mild systemic reaction at work with immediate onset of palpitations, impending doom and presyncope during preparation of an intravenous infusion of a PS80-containing amiodarone solution (Cordarone®). A skin prick test with an undiluted PEG 3350 (Movicol) solution was also positive at this time. The patient was diagnosed with a dual allergy to PEG and PS80 and was excluded from COVID-19 vaccination at that time. She tolerated both doses of the PEG-containing BNT162b2 vaccine using a graded challenge protocol 2 months later and tolerated a third graded challenge after 12 months.

**PEG 1 –** A 65-year-old female with a history of PR10-related oral allergy syndrome and cofactor-dependent hazelnut allergy was seen for an inpatient allergy consult 2 days after being hospitalised via the emergency department for severe anaphylaxis (hypotension with syncope, diarrhoea, abdominal cramps, absence of mucocutaneous symptoms) with transient tryptase elevation (32.5 µg/L versus 9.8 µg/L baseline) treated with epinephrine. The reaction occurred 5 minutes after intra-articular injection of a PEG 3350-containing methylprednisolone acetate with bupivacaine (Marcaine®) in the right knee. The patient was invited for outpatient testing 3 months after the index event at which time skin prick tests were positive for PEG 4000 (1/10), Depo-Medrol® (1/10) with negative prick and intradermal tests for

lidocaine, chlorhexidine and methylprednisolone succinate (Solu-Medrol®). Allele-specific qPCR for the somatic c-KIT D816V point mutation was negative in peripheral blood (genotyping for hereditary alpha-tryptasemia was not yet available at this time). She was diagnosed with a PEG allergy and advised to avoid all PEG-containing products. At a second outpatient visit, 6 months after the index event, an oral provocation test with methylprednisolone (Medrol) was tolerated up to a cumulative dose of 20.44 mg. Additional skin tests were positive at that time for PEG 4000 and 6000 but negative PEG 400 and PEG 1500 as well as for PS80. The patient was seen again 4 years later, for workup prior to COVID-19 vaccination, at which time an intradermal test with PS80 (1/10) was negative. Skin prick and intradermal test with Depo-Medrol® had also reverted to negative at this time. She tolerated 2 single-dose administrations of the PS80-containing Janssen vaccine 3 weeks and 6 months after this workup. She also tolerated a BNT162b2 booster administered through a graded vaccine challenge 9 months after the last Janssen dose.

**PEG 2 –** A 48-year-old male with allergic rhino-conjunctivitis due to grass pollen allergy was referred to the outpatient allergy clinic 5 weeks after an episode of severe anaphylaxis (generalized pruritus, hypotension) with transient tryptase elevation (23.8 µg/L versus 4.8 µg/L at baseline) treated with epinephrine. The reaction occurred immediately after intra-articular injection of a PEG 4000- and PS80-containing betamethasone dipropionate solution (Diprophos®) in the right elbow. The patient had tolerated an intra-articular methylprednisolone acetate (Depo-Medrol®) injection 3 years prior to the index event. The patient was seen 4 weeks later for outpatient skin testing which were positive for Depo-Medrol (1/10 IDT), Diprophos (1/1000 SPT and 1/100 IDT) and PEG 4000 (1 mg/mL SPT) and negative for methylpredinosolone succinate (Solu-Medrol®), hydrocortisone sodium succinate

(Solu-Cortef®), dexamethasone sodium phosphate (Aacidexam®), triamcinolone acetate (Volon®) and PEG 400. He was diagnosed with PEG allergy and advised to avoid all PEG-containing products. The patient was recalled for additional skin testing 9 years later prior to COVID-19 vaccination. Skin testing at that time was negative for PS80 and the skin test for PEG 4000 had also reverted to negative at that time. He tolerated 2 single-dose administrations of the PS80-containing Janssen vaccine 8 weeks and 6 months after this workup.

**PEG 3 –** A 33-year-old male without history of allergic disorders was referred to the outpatient allergy clinic 2 years after a mild anaphylactic episode (nausea and vomiting, pruritus, generalized urticaria) treated with oral and parenteral H1 antihistamines. The reaction occurred within 30 seconds after intra-articular injection of a PEG 3350-containing methylprednisolone acetate solution (Depo-Medrol®) with bupivacaine (Marcaine®) in the left knee. Intradermal skin testing with bupivacaine and methyl-prednisolone sodium succinate (Solu-Medrol®) were negative. Additional outpatient skin testing 3 months later with Depo-Medrol®, betamethasone and PEG 4000 did not result in local wheal-and-flare however did result in physician-observed generalized urticaria, erythema, nasal congestion and sneezing, treated with an oral H1 antihistamine. Repeat skin testing with PEG 4000 2 weeks later resulted in an identical systemic reaction, again without local wheal-and-flare. Additional oral provocation with methylprednisolone (Medrol®) 4 weeks later was tolerated up to a cumulative dose of 18 mg without any reaction. A placebo-controlled single blind skin test with PEG 4000, 3 months later, resulted in an identical systemic reaction to PEG but not to placebo (sterile water). The patient was diagnosed with PEG allergy and advised to avoid all PEG-containing products. He was recalled 5 years later for additional skin testing prior to COVID-19 vaccination. Intradermal skin testing at that

time was negative for PS80 and was not repeated for PEG. He tolerated 2 single-dose administrations of the PS80-containing Janssen and Novavax vaccines 8 weeks and 9 months after this workup, respectively.

**PEG 4 –** A 45-year-old female without history of allergic disorders was referred to the outpatient allergy clinic 3 months after a mild anaphylactic episode (nausea, vomiting, generalized urticaria and hoarseness) treated with parenteral H1 and H2 antihistamines and epinephrine aerosol. The reaction occurred immediately after intra-articular injection of a PEG 3350-containing methylprednisolone acetate solution (Depo-Medrol®) with bupivacaine (Marcaine®) in the right shoulder. Since the index event she underwent 3 lumbar infiltrations with lidocaine and dexamethasone sodium phosphate (Aacidexam®) without any reaction. Outpatient skin testing 3 weeks later were negative for chlorhexidine, latex, bupivacaine, methylprednisolone sodium succinate (Solu-Medrol®) and betamethasone (Celestone®). Skin testing with Depo-Medrol® did not result in a local wheal-and-flare reaction, however, within 10 minutes after intradermal injection of the 1/10 solution she experienced a mild physician-observed systemic reaction with pruritus, generalized urticaria and nasal congestion treated with an oral H1 antihistamine. Follow-up oral (Medrol) and intravenous (Solu-Medrol®) provocation testing after 3 months was well tolerated up to a cumulative dose of 8 and 22.2 mg, respectively. She was diagnosed with PEG allergy based on tolerated skin and provocation testing with different steroids as well atypical systemic reaction during skin testing with Depo-Medrol®, though allergy for methylprednisolone acetate could strictly speaking not be excluded. She was recalled 4 years later for additional skin testing prior to COVID-19 vaccination. Intradermal skin tests at this time were negative for both PS80 as well as PEG 4000.

She tolerated 2 single-dose administrations of the PS80-containing Janssen vaccine 4 weeks and 7 months after this workup.

**PEG 5 –** A 53-year-old female without history of allergic disorders was referred to the outpatient allergy clinic 3 years after a mild anaphylactic reaction (generalized pruritus, erythema and mild labial angioedema) within 15 minutes after starting oral intake of a PEG 3350-containing bowel prep (Moviprep®) with spontaneous resolution. She also reported a similar reaction (erythema, pruritus, tachycardia) immediately after injection of a PEG 3350-containing methylprednisolone acetate solution in the right trochanteric bursa several months prior to the consultation. Outpatient intradermal skin testing 3 weeks later was negative with methylprednisolone sodium succinate (Solu-Medrol®), hydrocortisone sodium succinate (Solu-Cortef®), betamethasone (Celestone®) and PEG 400. Intradermal skin testing with Depo-Medrol® and PEG 4000 did not result in a local wheal-and-flare reaction, however, several minutes after intradermal injection she experienced a physician-observed systemic reaction with discrete urticaria on the trunk, back and arms which spontaneously disappeared within 60 minutes. She was diagnosed with PEG allergy and advised to avoid all PEG-containing products. The patient was recalled 8 years later for additional skin testing prior to COVID-19 vaccination which were negative for PS80. PEG skin tests were not repeated at this time. She tolerated 2 single-dose administrations of the PS80-containing Janssen vaccine 2 weeks and 9 months after this workup.

**PEG 6 –** A 47-year-old male without history of allergic disorders was referred to the outpatient allergy clinic 20 months after an anaphylactic reaction (nausea, generalized erythema and urticaria, atrial fibrillation) treated with amiodarone. The reaction occurred within minutes after an intra-articular injection with a PEG 3350-

containing methylprednisolone acetate solution (Depo-Medrol®) with bupivacaine (Marcaine®) in the right elbow. Skin testing at that time was negative for latex, methylprednisolone sodium succinate (Solu-Medrol®) and bupivacaine (Marcaine®) and positive for Depo-Medrol® (IDT 1/1000) and PEG 4000 (SPT and IDT 1/1000). Additional skin testing with PEGs 6 weeks later was positive for PEG 6000 and negative for PEG 400 and 1500. He was contacted 5-years later and reported having tolerated recent vaccination with a PS80-containing influenza vaccine (Alfa-RIX Tetra) obviating the need for additional PS80 skin testing. He subsequently tolerated 3 single-dose administrations of a PS80-containing vaccine (2 doses Janssen vaccine, 2 and 8 months later and 1 dose of Nuvaxovid 14 months later). He also tolerated a BNT162b2 booster administered through a graded vaccination challenge 5 months after receiving Nuvaxovid. At time of booster vaccination, the patient reported having tolerated an oral PEG 4000-containing bowel prep 3 months prior.

**PEG 7 –** A 71-year-old female was referred to the outpatient allergy clinic 6 weeks after a generalized urticarial reaction without associated symptoms with onset within 2 hours after topical application of a PEG 400/3350-containing topical antibiotic cream (mupirocine, Bactroban®) on a superficial wound on the knee. She went to her general practitioner who prescribed a PEG 400/6000-containing oral H1 antihistamine (Loratadine Mylan) which she took thrice resulting in paradoxical worsening of the urticaria after each intake which finally subsided after substitution of the antihistamine with oral corticosteroids (Medrol®). The patient's general practitioner also reported a suspected (not well-described) allergic reaction immediately after intra-articular injection of a PEG 3350-containing methylprednisolone acetate solution (Depo-Medrol®) in the wrist, 7 years prior to first presentation. Skin prick tests 2 months after the index event were negative for

loratadine (Claritine) and mupirocine and intradermal tests were negative with methylprednisolone acetate and succinate (Depo-Medrol® and Solu-Medrol®) but positive with pure PEG 400 and PEG 4000 (1/10). The patient was diagnosed with PEG allergy and advised to avoid all PEG-containing products. She was contacted for additional workup prior to COVID-19 vaccination 11 years later but reported already having received and tolerated a single-dose administration of the PS80-containing Vaxzevria vaccine (AstraZeneca). She received 2 additional doses of PS80-containing vaccines (Vaxzevria and Janssen) 3 and 8 months later. She also received and tolerated a booster shot with the PEG-containing BNT162b2 vaccine though graded vaccination 10 months after the last Janssen dose.

**PS 1 –** A 46-year-old female with a history of breast cancer was referred to the outpatient allergy clinic after 2 anaphylactic episodes 2 months prior. The first episode (hypotension, desaturation, generalized erythema, abdominal cramping, diarrhoea) occurred within minutes after starting an IV infusion with a PS80-containing docetaxel solution (Taxotere). The reaction was treated with IV corticosteroids and H1-antihistamines and the chemotherapy regimen was subsequently switched to epirubicin-cyclophosphamide. Several weeks later, the day after the second chemotherapy cycle, she received a subcutaneous injection with a PEG 20,000- and PS20-containing rhGM-CSF solution (pegfilgrastim, Pelmeg) and immediately developed generalized urticaria, pruritus and hoarseness treated with IV corticosteroids and H1-antihistamines. Tryptase level obtained immediately after the reaction was slightly elevated though not reaching significance according to established guidelines (4.7 µg/L *versus* 2.4 µg/L baseline). Skin testing 4 weeks later was positive with PS80 (IDT 1/100), Taxotere (SPT 1/10) and negative for PEG 400 up to PEG 20,000. She also reported already having tolerated 2 single-dose

administrations of the PEG-containing BNT162b2 vaccine 4 months prior to the index event. The patient was diagnosed with an isolated PS80 allergy without PEG cross-reactivity and was advised to avoid all PS80-containing products. She received a single-dose booster shot with BNT162b2 10 months later without any reaction.

**PS 2 –** A 50-year-old female with a history of acute lymphoid leukemia treated with autologous bone marrow transplantation, myasthenia gravis and rheumatoid arthritis was seen for an inpatient allergy consult while hospitalised at the neurology department after an allergic reaction (angioedema, wheezing and hypotension) 1 hour after initiation of the sixth infusion of a PS80-containing anti-CD20 monoclonal antibody (rituximab) for myasthenia gravis. Tryptase was not significantly elevated immediately after the reaction (5.9 µg/L versus 4.8 µg/L at baseline). She had previously received 5 rituximab infusions and reported having tolerated the first 4 without problems but developing generalized urticaria 24 hours after the fifth infusion treated with oral H1 antihistamines and corticosteroids. Due to the absence of tryptase elevation and atypical presentation, a pseudo-allergic rather than IgE-mediated reaction was suspected at first. Due to clinical need, the next rituximab administration was performed under allergist supervision according to a rapid desensitization protocol. This resulted in severe symptomatic bronchoconstriction (> 50% PEF reduction) at the penultimate desensitization step, treated with IV corticosteroids, H1 antihistamines and inhaled beta-2 agonists. A slower 12-step desensitization protocol was tolerated without any reaction 1 week later. Two additional administrations occurred according to this desensitization protocol over the next month with the patient reporting delayed onset of self-limiting generalized urticaria 24 hours after each treatment. The patient presented at the neurology department 8 months later due to recurrence of myasthenia symptoms. She also

reported having suffered an immediate severe anaphylactic reaction (urticaria, angioedema, wheezing, hypotension) after administration of a first dose of the PS80-containing COVID-19 vaccine (Vaxzevria) 1 month prior. Skin testing 1 week later was positive for PS80 (SPT 1/1) and negative for PEG 3350 (IDT 1/10) and PEG 20,000 (SPT 1/1). She was diagnosed with an isolated PS80 allergy and tolerated 2 single-dose administrations of the PEG-containing BNT162b2 vaccine 1 and 7 months later. She also tolerated an intravenous administration of a PS80-free anti-CD20 monoclonal (obinutuzumab, Gazyvaro®) which contained the (PEG-based) excipient poloxamer 188.

## Tables

**Table E1.** Graded vaccination challenge (GVC) protocol with BNT162b2

| Step | Time | Preparation | Dose† (% of normal dose) | Cumulative dose (% of normal dose) |
|---|---|---|---|---|
| 1 | 0 minutes | 0.05 mL of 1/100 vaccine dilution in sterile water | 0.05 µg (0.17%) | 0.05 µg (0.17%) |
| 2 | 15 minutes | 0.05 mL of 1/10 vaccine dilution in sterile water | 0.5 µg (1.67%) | 0.551 µg (1.84%) |
| 3 | 30 minutes | 0.05 mL of undiluted vaccine | 5 µg (16.67%) | 5.551 µg (18.51%) |
| 4 | 45 minutes | 0.10 mL of undiluted vaccine | 10 µg (33.33%) | 15.55 µg (61.84%) |
| 5 | 60 minutes | 0.15 mL undiluted vaccine | 15 µg (50%) | 30.55 µg (101.84%) |

† Dose is reported as active ingredient (mRNA tozinameran) in µg

**Table E2.** Timing of diagnostic evaluation and sample collection

| ID | Index event – ST (tested agent, result) | | Exposures post-index event, pre-vaccination (trigger, reaction) | x – First COVID-19 vaccine dose | | | Index – Sampling (result) | |
|---|---|---|---|---|---|---|---|---|
| | Initial ST | Repeat ST | | x = Index event (type, reaction) | x = Last PEG ST (result) | x = Last PS80 ST (result) | Serum (PEG sIgE) | wb-BAT (BNT162b2) |
| DA1 | 1m (PEG, +) | 11y1m (PS80, +) | PEG (multiple, +) | 11y8m (PEG GVC, -) | 11y7m (+) | 7m (+) | 11y1m (+) | 11y8m (+) |
| DA2 | 2m (PEG, +) | 10y (PS80, +) | PEG (multiple, +) | 10y10m (PEG GVC, -) | 10y8m (+) | 8m (+) | 10y (+) | 10y8m (+) |
| DA3 | 4y (PS80, +)<br>4y2m (PEG, +) | np | PEG (multiple, +)<br>PS80 (single, +) | 4y4m (PEG GVC, -) | 2m (+) | 4m (+) | 4y (+) | 4y4m (+) |
| PEG1 | 3m (PEG, +)<br>6m (PS80, -) | 4y6m (PEG, -)<br>4y6m (PS80, -) | None | 4y7m (PS80 SD, -) | 1m (-) | 1m (-) | 4y6m (-) | 6y8m (+) |
| PEG2 | 2m (PEG, +) | 9y (PEG, -)<br>9y (PS80, -) | None | 9y2m (PS80 SD, -) | 2m (-) | 2m (-) | 9y (np)† | np |
| PEG3 | 2y3m (PEG, +) | 7y2m (PS80, -) | None | 7y4m (PS80 SD, -) | 5y1m (+) | 2m (-) | 7y2m (-) | np |
| PEG4 | 4m (PEG, +) | 4y7m (PEG, -)<br>4y7m (PS80, -) | None | 4y8m (PS80 SD, -) | 1m (-) | 1m (-) | 4y7m (-) | np |
| PEG5 | 3y (PEG, +) | 13y (PS80, -) | PEG (single, +) | 13y1m (PS80 SD, -) | 10y (-) | 1m (-) | 13y (+) | np |
| PEG6 | 1y8m (PEG, +) | np | PEG (single, -)<br>PS80 (single, -) | 6y10m (PS80 SD, -) | 5y2m (-) | np§ | 8y (-) | 8y (+) |
| PEG7 | 2m (PEG, +) | np | PS80 (single, -) | 11y (PS80 SD, -) | 10y10m (-) | np§ | 12y6m (-) | 12y6m (-) |
| PS1 | 3m (PEG, -)<br>3m (PS80, +) | np | None | 10m (PEG SD, -) | 7m (-) | 7m (+) | 10m (-) | 10m (-) |
| PS2 | 8m (PEG, -)<br>8m (PS80, +) | np | PS80 (multiple, +) | 7m (PS80 SD, +) | -1m (-) | -1m (+) | 9m (-) | 2y (-) |

Intervals are indicated as years (y) and months (m) elapsed since reference event (index event or x). Nature and outcome of exposures are indicated between parentheses as positive (+) or negative (-).

† Serum was used for both allo-BAT and PEG-sIgE measurement however in PEG2 remaining volume after allo-BAT was insufficient to allow for additional measurement of PEG-specific IgE. § PS80 skin testing was not performed in PEG6 and PEG7 since *in vivo* tolerated PS80 exposure was confirmed (Influenza vaccine in PEG6 and Vaxzevria in PEG7).

*Abbreviations:* ST, skin test; sIgE, specific IgE; wb-BAT, whole blood basophil activation test; y, years; m, months; GVC, graded vaccine challenge; SD, single-dose vaccination; np, not performed.

**Table E3.** Biochemical characteristics of excipient allergic subjects

| | Peripheral blood | | | | | | | | Other parameters | | | | | | Specific IgE (kU/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RBC ($x10^9$/L) | PLT ($x10^9$/L) | WBC ($x10^{12}$/L) | Neu (%) | Eos (%) | Bas (%) | Ly (%) | Mon (%) | CRP (mg/L) | IgG (g/L) | IgM (g/L) | IgA (g/L) | Tryptase (µg/L) | Total IgE (kU/L) | House dust mite (d1) | Grass pollen mix (gx3) | Tree pollen mix (tx10) | Cat dander (e1) | Dog dander (e5) | Alternaria (m6) | Mugwort (w6) |
| *Reference* | *4.5-6* | *150-400* | *4-10* | *40-70* | *0-5* | *0-3* | *21-41* | *0-12* | *< 5* | *7,51-15,60* | *0,46-3,04* | *0,82-4,53* | *< 11* | *< 114* | *< 0,10* | *< 0,10* | *< 0,10* | *< 0,10* | *< 0,10* | *< 0,10* | *< 0,10* |
| DA1 | 5,3 | 274 | 7,29 | 42,6 | 5,6 | 1,4 | 39,9 | 10,2 | 0,3 | 7,37 | 1,24 | 1,53 | 3,8 | 3403 | 5,82 | 0,17 | 0,3 | < 0,10 | 0,31 | 0,17 | 0,64 |
| DA2 | 5,1 | 262 | 5,09 | 56,1 | 2,2 | 0,6 | 34,8 | 6,3 | 2 | 9,7 | 0,54 | 3,22 | 5,3 | 152 | 22,2 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| DA3 | 4,5 | 258 | 8,05 | 51,7 | 1,5 | 0,2 | 38,9 | 7,3 | 1.2 | 10,9 | 1,74 | 2,77 | 4,3 | 296 | 0,33 | < 0,10 | < 0,10 | < 0,10 | 0,14 | < 0,10 | < 0,10 |
| PEG1 | 4,62 | 180 | 5,4 | 50,9 | 2,6 | 0,6 | 38,3 | 7,6 | 1,4 | 12,7 | 4,32 | 2,93 | 9,8 | 13,8 | < 0,10 | < 0,10 | 1,35 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| PEG2 | 4,96 | 249 | 4,72 | 57,9 | 2,1 | 0,4 | 32,8 | 6,8 | 1,1 | 11,9 | 0,66 | 2,64 | 4,8 | 22,5 | < 0,10 | 8,9 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| PEG3 | 4,8 | 225 | 5,66 | 62,6 | 2,3 | 0,4 | 34,6 | 10,1 | 7,6 | na | na | na | 6,3 | 74 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| PEG4 | 5,07 | 282 | 8,83 | 65 | 1,6 | 0,6 | 27,4 | 5,4 | 2,2 | na | na | na | 4,7 | 169 | na | na | na | na | na | na | na |
| PEG5 | 5,11 | 194 | 6,15 | 52,7 | 3,7 | 0,5 | 35,9 | 7,2 | 0,7 | na | na | na | 2,8 | 1778 | na | na | na | na | na | na | na |
| PEG6 | 4,67 | 118 | 4,4 | 54,9 | 1,9 | 0,8 | 35,2 | 7,2 | 0,6 | na | na | na | 4,4 | 29 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| PEG7 | 4,32 | 237 | 6,37 | 67,6 | 2 | 0,6 | 22,4 | 7,4 | 2 | na | na | na | 7,3 | 70 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |
| PS1 | 4,83 | 246 | 5,3 | 64,5 | 2,1 | 0,6 | 27 | 5,8 | 1,3 | na | na | na | 2,4 | 1955 | 0,21 | 20,5 | < 0,10 | < 0,10 | 0,27 | < 0,10 | 1,95 |
| PS2 | 4,7 | 252 | 6 | 54,1 | 2 | 0,5 | 29,2 | 14 | 0,6 | 6,51 | 0,3 | 1,84 | 4,8 | 22 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 | < 0,10 |

*Abbreviations:* RBC, red blood cell count; PLT, platelet count; WBC, white blood cell count; Neu, % neutrophils (of total WBC); Eos, % eosinophils (of total WBC); Bas, % basophils (of total WBC); Ly, % lymphocytes (of total WBC); Mon, % monocytes (of total WBC); na, not available.

505 **Table E4.** Characteristics of anaphylaxis controls

| ID | Sex | Age (y) | Total IgE (kU/L) | Anaphylaxis | Known COVID-19 vaccination history | Sampling prior to first vaccine dose? |
|---|---|---|---|---|---|---|
| AC1 | M | 38 | 22 | Cephazolin | 3x BNT162b2<br>1x mRNA-1273 | Yes |
| AC2 | F | 64 | 28 | Wasp venom | 2x Vaxzevria<br>3x BNT162b2 | Yes |
| AC3 | F | 22 | 47 | Wasp venom | 2x Vaxzevria<br>2x BNT162b2 | Yes |
| AC4 | M | 74 | 134 | Beer yeast | 3x Vaxzevria<br>2x BNT162b2 | Yes |
| AC5 | M | 41 | 202 | Horsefly saliva | 2x Pfizer<br>1x mRNA-1273 | No |
| AC6 | M | 64 | 376 | Wheat (WDEIA) | 3x BNT162b2<br>1x mRNA-1273 | Yes |
| AC7 | F | 22 | 528 | Idiopathic | 3x BNT162b2<br>1x mRNA-1273 | No |
| AC8 | M | 24 | 748 | Food (poly-allergy) | 3x BNT162b2<br>1x mRNA-1273 | Yes |
| AC9 | F | 19 | 853 | Salmon | 2x BNT162b2<br>1x mRNA-1273 | Yes |
| AC10 | M | 47 | 1062 | Wasp venom | 2x BNT162b2<br>1x mRNA-1273 | Yes |
| AC11 | M | 35 | 1719 | Latex | 3x mRNA-1273<br>1x BNT162b2 | Yes |
| AC12 | M | 60 | 2305 | Wasp venom | 1x Ad26.COV2.S<br>3x BNT162b2 | No |
| AC13 | M | 48 | 3289 | Wasp venom | 5x BNT162b2 | Yes |
| AC14 | M | 49 | > 5000 | Food (poly-allergy) | 2x BNT162b2 | No |
| AC15 | F | 19 | > 5000 | Food (poly-allergy) | 2x BNT162b2<br>1x mRNA-1273 | Yes |

506 *Abbreviations*: WDEIA, wheat-dependent exercise-induced anaphylaxis

507 **Table E5.** Characteristics of healthy controls

| ID | Sex | Age (y) | Allergy | Vaccination status† | COVID-19 status§ |
|---|---|---|---|---|---|
| HC1 | F | 43 | - | BNT162b2 (3x) | naive |
| HC2 | F | 25 | - | Vaxzevria (2x)<br>BNT162b2 (1x) | positive, -4 weeks |
| HC3 | F | 28 | ARC (house dust mite) | BNT162b2 (3x) | positive, -6 weeks |
| HC4 | F | 58 | ARC (house dust mite, birch pollen) | BNT162b2 (3x) | positive, -10 weeks |
| HC5 | M | 29 | - | BNT162b2 (3x) | naive |
| HC6 | F | 58 | - | BNT162b2 (3x) | naive |

508 † All controls received and tolerated a dose of BNT162b2 < 4 months prior to
509 sampling. § PCR-proven SARS-CoV-2 infection, interval between positive test and
510 sampling. ARC, allergic rhinoconjunctivitis.

511

512 **Table E6.** Reagents for basophil activation testing (BAT)

| **Reagent** | **Manufacturer**<br>*Category no°* | **Concentration**<br>*Application* | **Notes** |
|---|---|---|---|
| Basophil Stimulation Buffer (BSB) | In-house formulation | - | 20 mM HEPES; 133 mM NaCl; 5 mM KCl; 7.5 mM $CaCl_2$; 3.5 mM $MgCl_2$; 0.1% HSA (w/v); 0.5 mM glucose; pH 7.4 |
| Recombinant human interleukin-3 (rhIL-3) | PeproTech<br>*200-03* | 120 ng/mL | Fresh rhIL-3 added to complete BSB (cBSB) prior to experiment |
| Polyclonal goat anti-human IgE (aIgE) | Sigma-Aldrich<br>*I6284* | 5 µg/mL | IgE-dependent positive control |
| N-formyl-Met-Leu-Phe (fMLP) | Sigma-Aldrich<br>*F3506* | 2 µM | IgE-independent positive control |
| Dasatinib (das) | Toronto Research Chemicals<br>*D193600* | 0.01 – 1 µM | Stock solution (1 mM) in dimethylsulfoxide (DMSO) |
| BNT162b2 (Comirnaty®) | Pfizer-BioNTech | 0.8 - 50 µg/mL<br>*wb/allo-BAT*<br>50 µg/mL<br>*allo-BAT inh.* | Remnants of freshly prepared vaccines (100 µg/mL in USP), otherwise meant to be disarded, used < 6 hours or stored at -20°C until use[†]; dissolved in cBSB for BAT |
| ALC-0159 | BroadPharm<br>*BP-25711* | 3.4 – 400 µg/mL<br>*wb-BAT*<br>8 – 200 µg/mL<br>*allo-BAT* | Stock solution (10 mg/mL) in N,N-dimethylformamide (DMF)[$]; dissolved in cBSB |
| PEG 20,000 | Sigma-Aldrich<br>*25322-68-3* | 0.16 – 50 mg/mL<br>*wb-BAT*<br>20 mg/mL<br>*allo-BAT* | Stock solution (100 mg/mL) in NaCl 0.9%; dissolved in cBSB for BAT |
| PEG 4000 | Fagron<br>*15272-95-4*<br>Sigma-Aldrich<br>*95904* | 0.16 – 50 mg/mL<br>*wb-BAT, allo-BAT inh*<br>50 mg/mL<br>*allo-BAT* | Stock solution (100 mg/mL) in sterile water for injection; dissolved in cBSB for BAT<br>Stock solution (100 mg/mL) in NaCl 0.9%; dissolved in cBSB for BAT |
| PEG 400 | Fagron<br>*9002-92-0* | 50 mg/mL<br>*allo-BAT inh.* | Stock solution (undiluted) diluted 1:2 in NaCl 0.9% |
| Diethylene glycol (DEG) | Sigma-Aldrich<br>*H26456* | 10 mg/mL<br>*allo-BAT inh.* | Stock solution (1.1 g/mL); dissolved in PBS to 100 mg/mL |
| Polysorbate 80 (PS80) | Fagron<br>*9005-65-6*<br>Sigma-Aldrich<br>*P1754* | 1.6 – 500 ng/mL<br>*wb-BAT (BFD)*<br>0.16 – 50 mg/mL<br>*wb-BAT*<br>50 mg/mL<br>*allo-BAT inh.* | Stock solution (1 mg/mL) in sterile water for injection; dissolved in cBSB for BAT<br>Stock solution (1 mg/mL or 100 mg/mL) in NaCl 0.9%); dissolved in cBSB for BAT |
| House dust mite (HDM) extract | Greer<br>*NC9756554* | 0.01 – 100 ng/mL<br>*allo-BAT inh.* | Stock solution (2 mg/mL) in NaCl 0.9% |
| Anti-CD123<br>PE | BioLegend<br>*306006* | 1:10 in PBS | Mouse IgG1 kappa<br>Clone 6H6 |
| Anti-HLA-DR<br>AF 647 | BioLegend<br>*307622* | 1:10 in PBS | Mouse IgG2a kappa<br>Clone L243 |

| | | | |
|---|---|---|---|
| Anti-CD63 FITC | BioLegend *353006* | 1:10 in PBS | Mouse IgG1 kappa Clone H5C6 |
| FACS lysis buffer | BD *349202* | - | Stock solution 10x dissolved in sterile water |
| Paraformaldehyde (PFA) | - | 1% in sterile water | Fixating agent |
| CHAPS | Thermo Scientific *B21927.06* | 0.005 – 0.5% in BSB *allo-BAT* | Dissolved in BSB; BSB-CHAPS was then used to dilute stimuli (i.e. BNT162b2, HDM extract) |

*Abbreviations:* BAT, basophil activation testing; BSB, basophil stimulation buffer; HSA, human serum albumin; rhIL-3, recombinant human interleukin-3; aIgE, anti-IgE; fMLP, N-formyl-methionyl-leucyl-phenylalanine; Das, dasatinib; DMSO, dimethylsulfoxide; wbBAT, whole blood BAT; allo-BAT inh., allo-BAT inhibition; BFD, before first dose; cBSB, complete basophil stimulation buffer (BSB + rh-IL-3 120 ng/mL); DMF, N,N-dimethylformamide; PEG, polyethylene glycol; PS80, polysorbate 80: PE, phycoerythryin; AF 647, Alexa Fluor 647; FITC, Fluorescein Isothiocyanate; PFA, paraformaldehyde.

† Stability of BNT162b2 in fresh vaccine samples (< 6h after preparation) versus samples stored for 2 weeks at -20°C and thawed prior was assessed through concurrent BAT on fresh blood sample (DA1) with vaccine from both storage conditions eliciting identical dose-response curves.

$ Toxicity of ALC-0159 in N,N-dimethylformamide (DMF) was assessed through incubation of fresh nonallergic donor blood with anti-IgE and various concentrations of ALC-0159 stock solution in DMF (10 mg/mL), diluted in BSB, showing decrease of basophil responses to anti-IgE with ALC-0159 concentrations above 400 µg/mL.

**Table E7.** Basophil reactivity to tested antigens in whole blood of patients and controls

| | | Basophil reactivity (max % C63+ basophils)† | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **anti-IgE R** | **fMLP R** | **BNT162b2** | **ALC-0159** | **PEG 20,000** | **PEG 4000** | **PS80** |
| Dual-allergic | DA1 | yes | yes | 95.7 | np | np | 32.3 | 12.5 |
| | DA2 | yes | yes | 86.8 | 4.5 | np | 1.5 | 4.1 |
| | DA3 | yes | yes | 80.9 | 28 | 0 | 16.7 | 13.6 |
| Previously PEG mono-allergic | PEG1 | yes | yes | 76.1 | 4.1 | 1.3 | 47 | 2.3 |
| | PEG6 | yes | yes | 17.2 | 1 | 1.3 | 0 | 0 |
| | PEG7 | yes | yes | 0 | 0 | 0.8 | 1.3 | 0.1 |
| PS80 mono-allergic | PS1 | yes | yes | 2.8 | np | np | 1 | 3 |
| | PS2 | no (1.7%) | yes | 0.2 | 0 | 1.5 | 0 | 0.3 |
| Healthy controls | HC1 | yes | yes | 0,7 | 0.1 | np | np | np |
| | HC2 | yes | yes | 1.9 | 1.4 | np | np | np |
| | HC3 | yes | yes | 1.7 | 0.8 | 0.4 | 0.7 | 1.1 |
| | HC4 | yes | yes | 0.6 | 1.3 | np | 0.9 | 0 |
| | HC5 | yes | yes | 0 | 0.4 | 0.5 | 0 | 0.1 |
| | HC6 | yes | yes | 0.6 | 1.2 | np | Np | np |

† Reactivity expressed as maximum % $CD63^+$ basophils across all tested concentrations and timepoints.

*Abbreviations*: R, responder (> 10% $CD63^+$ basophils to anti-IgE or fMLP); np, not performed

**Table E8.** Donor basophil reactivity to tested antigens in allo-BAT after passive sensitization with serum of patients and controls

| | | Basophil reactivity (max % C63+ basophils)† | | | | |
|---|---|---|---|---|---|---|
| | | **BNT162b2** | **ALC-0159** | **PEG 20,000** | **PEG 4000** | **PS80** |
| Dual-allergic | DA1 | 29.8 | 25.8 | 0 | 0.9 | 4.2 |
| | DA2 | 23.2 | 11.8 | 1.9 | 0.6 | 0.3 |
| | DA3 | 49.9 | 16.4 | 0 | 0 | 4.5 |
| Previously PEG mono-allergic | PEG1 | 5.3 | 11.1 | 0.3 | 2.2 | 0 |
| | PEG2 | 1.3 | 4.5 | 0 | 0 | 0 |
| | PEG3 | 2.8 | 9.9 | 0 | 0 | 0 |
| | PEG4 | 1.5 | 5.3 | 0.6 | 0 | 0 |
| | PEG5 | 4.2 | 3.8 | 0 | 0 | 0.1 |
| | PEG6 | 3.7 | 0.3 | 0 | 0 | 0 |
| | PEG7 | 3.6 | 1.2 | 0 | 0 | 0 |
| PS80 mono-allergic | PS1 | 0.9 | 1 | np | np | 6 |
| | PS2 | 0 | 1.4 | np | np | 1.4 |
| Healthy controls | HC1 | 0 | 3.5 | 0.6 | 0 | 1.2 |
| | HC2 | 0.1 | 4.5 | 1.2 | 0.2 | 0 |
| | HC3 | 0 | 4.5 | 0 | 0 | 0.8 |

† Reactivity expressed as maximum % $CD63^+$ basophils across all tested concentrations and timepoints.

*Abbreviations*: np, not performed

**Table E9.** Total and PEG-specific IgE levels directly measured in serum of patients and controls (ImmunoCAP™)

| | | Total IgE† | PEG 2000 sIgE$ | | PEG 10,000 sIgE$ | | wb-BAT | |
|---|---|---|---|---|---|---|---|---|
| | | kU/L | kU/L | RU | kU/L | RU | BNT162b2 | PEG 4000 |
| Dual-allergic | DA1 | 3403 | > 100 | 19639 | > 100 | 21471 | +++ | ++ |
| | DA2 | 152 | 0.66 | 187 | 0.94 | 264 | +++ | - |
| | DA3 | 296 | 0.79 | 221 | 1.08 | 302 | +++ | + |
| Previously PEG mono-allergic | PEG1 | 13.3 | < 0.1 | 13 | < 0.1 | 10 | ++ | +++ |
| | PEG3 | 74 | < 0.1 | 15 | < 0.1 | 13 | np | np |
| | PEG4 | 169 | < 0.1 | 26 | < 0.1 | 25 | np | np |
| | PEG5 | 1778 | 0.74 | 209 | 0.7 | 199 | np | np |
| | PEG6 | 29 | < 0.1 | 9 | < 0.1 | 11 | + | - |
| | PEG7 | 70 | < 0.1 | 14 | < 0.1 | 13 | - | - |
| PS80 mono-allergic | PS2 | 22 | < 0.1 | 10 | < 0.1 | 10 | - | - |
| Healthy controls | HC1 | np | < 0.1 | 9 | < 0.1 | 9 | - | - |
| | HC2 | np | < 0.1 | 9 | < 0.1 | 9 | - | - |
| | HC3 | np | < 0.1 | 10 | < 0.1 | 8 | - | - |
| | HC4 | np | < 0.1 | 11 | < 0.1 | 13 | - | - |
| | HC5 | np | < 0.1 | 13 | < 0.1 | 9 | - | - |
| | HC6 | np | < 0.1 | 10 | < 0.1 | 10 | - | - |
| Anaphylaxis controls | AC1 | 22 | < 0.1 | 11 | < 0.1 | 10 | np | np |
| | AC2 | 18 | < 0.1 | 10 | < 0.1 | 9 | np | np |
| | AC3 | 47 | < 0.1 | 11 | < 0.1 | 10 | np | np |
| | AC4 | 134 | < 0.1 | 12 | < 0.1 | 11 | np | np |
| | AC5 | 202 | < 0.1 | 20 | < 0.1 | 17 | np | np |
| | AC6 | 376 | < 0.1 | 15 | < 0.1 | 13 | np | np |
| | AC7 | 528 | < 0.1 | 16 | < 0.1 | 16 | np | np |
| | AC8 | 748 | < 0.1 | 24 | < 0.1 | 19 | np | np |
| | AC9 | 853 | < 0.1 | 17 | < 0.1 | 15 | np | np |
| | AC10 | 1062 | < 0.1 | 23 | < 0.1 | 21 | np | np |
| | AC11 | 1719 | < 0.1 | 36 | < 0.1 | 27 | np | np |
| | AC12 | 2305 | < 0.1 | 35 | < 0.1 | 31 | np | np |
| | AC13 | 3289 | 0.13 | 43 | 0.11 | 38 | np | np |
| | AC14 | > 5000 | 0.19 | 60 | 0.18 | 57 | np | np |
| | AC15 | > 5000 | 0.34 | 101 | 0.24 | 73 | np | np |

† Upper reporting limit of total IgE assay is 5000 kU/L. Assay performed in the clinical laboratory of University Hospitals Leuven.

$ Upper reporting limit of specific IgE assays is 100 kU/L, lower reporting limit is 0.1 kU/L. Assay performed at research laboratory of Phadia, Uppsala, Sweden.

549 *Abbreviations*: sIgE, specific IgE; wb-BAT, whole blood BAT; RU, arbitrary
550 fluorescence response units; np, not performed.

551

552 **Figures**

553 **FIG E1.** Schematic size comparison of ethylene glycol-based excipients and size
554 requirements for IgE cross-linking. 'n' denotes number of ethylene glycol moieties
555 present in each molecule; bottom panel shows the minimal size requirements of
556 divalent allergens for cross-linking of adjacent receptor-bound IgE.[E9] * ALC-0159
557 micelle diameter was inferred from Wu et al.[E10] ; δ BNT162b2 LNP size was inferred
558 from Kudsiova et al.[E11] LNP, lipid nanoparticle.

559 *Abbreviations:* DEG, diethylene glycol; PEG, polyethylene glycol; LNP, lipid
560 nanoparticle.

561 **FIG E2.** Flowcytometric gating strategy for BAT experiments. Gating shown for
562 representative allo-BAT experiment with serum of DA1. Gating was determined on
563 sample stimulated with basophil stimulation buffer (BSB) and applied to other
564 samples. Blue arrow indicates direction of sequential gating: exclusion of aggregates
565 > selection of singlets based on forward (FSC) and side scatter (SSC) area (A) and
566 height (H) > selection of basophils (CD123+/HLA-DR-) > selection of activated
567 basophils (CD63+).

568 **FIG E3.** Whole blood basophil activation test (wb-BAT) results (% $CD63^+$ basophils)
569 after stimulation with control stimuli (anti-IgE and fMLP) and dilution series of
570 BNT162b2, polyethylene glycol (PEG) 4000, polysorbate 80 (PS80) and ALC-0159 in
571 fresh blood samples of dual-allergic patients (DA1-3). Samples were obtained at
572 different timepoints in relation to the graded vaccine challenges with BNT162b2.
573 Each dot represents a single measurement.

574 *Abbreviations:* anti-IgE, polyclonal goat anti-human IgE; fMLP, formyl-Leucyl-
575 Methionyl-Phenylalanine; BFD, before first dose; AFD, after first dose; BSD, before

second dose; 7wASD, 7 weeks after second dose; 7m ASD, 7 months after second dose.

**FIG E4.** Whole blood basophil activation test (wb-BAT) results (% $CD63^+$ basophils) after stimulation with control stimuli (anti-IgE and fMLP) and dilution series of BNT162b2, ALC-0159, polyethylene glycol (PEG) 20,000, PEG 4000 and polysorbate 80 (PS80) in fresh blood samples of previously PEG mono-allergic patients (n=3). Samples were obtained at a single timepoint for each patient, immediately prior to graded vaccine challenges with BNT162b2. Each dot represents a single measurement.

*Abbreviations:* anti-IgE, polyclonal goat anti-human IgE; fMLP, formyl-Leucyl-Methionyl-Phenylalanine.

**FIG E5. A,** allo-BAT experiment using serum of patient DA3. Serum was left unaltered (standard allo-BAT, black triangles), heat-inactivated for 30' at 56°C (brown circles), or heat-inactivated and subsequently reconstituted through addition of unaltered non-allergic serum of HC5 (purple squares) prior to passive sensitization of stripped donor basophils. **B,** comparison of allo-BAT (continuous lines) and wb- BAT (black circles, dotted line) responses to wasp venom (0.01-10 µg/mL, ALK-Abelló, Hørsholm, Denmark) in serum compared to fresh blood of the same wasp venom allergic patient. Allo-BAT was performed in presence or absence of rhIL-3 in the stimulation buffer and with both heat-inactivated or unaltered serum for passive sensitization.

**FIG E6.** Allo-BAT experiments (n=2) using donor basophils of HC5 sensitized with serum of DA2 and DA3 and stimulated with BNT162b2 (20 µg/mL). Both patient sera were preincubated with either PBS (wo/ preincubation), or PEG 400 (w/

preincubation) or PS80 (w/ preincubation). Donor basophils were either washed and reconstituted in donor serum prior to stimulation (w/ washing) or left in patient serum (wo/ washing). Basophil responses are indicated as mean % CD63+ basophils (broad bars) with standard error (error bars). Experiments with each inhibitor were performed on 2 separate timepoints using the same patient sera and basophil donor. No inhibition was observed on preincubation of serum with PEG 400 and stimulation with house dust mite extract (DA2 only, data not shown).

**FIG E7.** Effect of dasatinib pretreatment on whole blood BAT responses to various IgE-dependent and IgE-independent stimuli. Experiment was performed on a fresh whole blood sample obtained from a birch pollen and house dust mite allergic control. Interleukin-3 containing stimulation buffer was used as negative control (HC4).

*Abbreviations*: BP, birch pollen; HDM, house dust mite; fMLP, formyl-Leucyl-Methionyl-Phenylalanine; PMA, phorbol-myristic acid.

**FIG E8.** Serum PEG-specific IgE measured through an ImmunoCAP™ fluorescent-enzyme-immunoassay with ethylene glycol-free wash buffer. **A**, specific IgE towards PEG 2000 expressed in RU measured in serum of anaphylaxis controls (n=15, inverted green triangles), dual-allergic (n=3, open squares), previously PEG mono-allergic (n=6, closed squares), PS80 mono-allergic (n=1, blue circle) (y-axis) *versus* corresponding total IgE values in kU/L (x-axis). **B**, relative decrease (% inhibition, leftward grey bars) or absolute decrease (RU, rightward red bars) of specific IgE values towards PEG 2000 measured in serum of DA1-3 and AC13-14 after preincubation with BNT162b2 at a 10% v/v ratio *versus* PBS 10%. Table under graph contains absolute RU values for each condition.

623 All values reported are means of duplicate experiments, expressed in either in kilo-
624 units per liter (kU/L) or arbitrary fluoresence response units (RU). Horizontal dotted
625 line indicates standard threshold of positivity utilized by the manufacturer (0.35 kU/L
626 corresponding with 106 RU).

627 *Abbreviations:* sIgE, specific IgE; ULR, upper limit of reporting; LLR, lower limit of
628 reporting; RU, response units; DA, dual-allergic; AC, anaphylaxis control.

629

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