Online supplement

Sensitisation to recombinant *Aspergillus fumigatus* allergens and clinical outcomes in COPD

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SUPPLEMENTARY MATERIALS AND METHODS

Ethics approval: Written informed consent was obtained from all recruited participants and Institutional ethics approval from each site obtained as follows: CIRB 2017/2933 and CIRB 2017/2109 (all mutually recognized by DSRB, Singapore), UMMC 2018725-6524 (Malaysia), CREC 2011.146, CREC 2015.164 and CREC 2018.042 (Hong Kong). Non-diseased (control) recruitment was approved by the Nanyang Technological University (NTU) Institutional Review Board under IRB-2017-12-010 (Singapore).

Blood sampling: Venous blood was collected from each participant at recruitment following informed consent. Plasma was isolated with centrifugation at 1300g for 10 minutes at 18°C and stored at -80 degrees prior to immune-dot-blot assay assessment as described. All specimens from clinical sites were transported (temperature controlled) and processed centrally in Singapore to ensure consistency and standardization of all assessments.

Allergen panel: House dust mite (*Dermatophagoides farinae* (Der f); *Dermatophagoides pteronyssinus* (Der p); *Blomia tropicalis* (Blo t)), pollens (*Elaeis guineensis*; Panicoids (Johnson grass (*Sorghum halepense*)); Pooids (Timothy grass (*Phleum pratense*); Meadow fescue (*Festuca pratesis*); Perennial ryegrass (Lolium perenne)); Chloroids (Bermuda grass (*Cynodon dactylon*)); Weeds (*Brassica spp, Ambrosia artemisiifolia, Helianthus annuus*) and cockroach (*Blattella germanica; Periplaneta Americana)* were included. A comprehensive panel of crude and recombinant fungal allergens included are summarized in Table 2.

Immune-dot-blot assay: Specific IgE to crude and recombinant allergens from the allergen panel were assessed using immune-dot-blot assays as previously described [1-3]. Crude allergens included: *Dermatophagoides farinae* (Der f) *Dermatophagoides pteronyssinus* (Der p), *Blomia tropicalis* (Blo t), *Elaeis guineensis*, Panicoids, Pooids, Chloroids, Weeds, *Blattella germanica* (Bla g), *Periplaneta Americana*, *Curvularia*, *Penicillium*, *Aspergillus fumigatus* (*A.fumigatus*), *A. terreus*, *A. sydowii*, *Cladosporium tenuissimum, Cladosporium* spp., *Neurospora* spp., *Byssochlamys spectabilis*, *Trametes sanguinea* and *Schizophyllum commune*. Recombinant allergens included Asp f 1 (M83781), Asp f 2 (U56938), Asp f 3 (U58050) ,Asp f 4 (AJ001732), Asp f 5 (Z30424), Asp f 6 (U53561), Asp f 7 (AJ223315), Asp f 9 (AJ223327), Asp f 10 (X85092), Asp f 11 (AJ006689), Asp f 12 (U92465), Asp f 13 (Z11580), Asp f 15 (AJ002026), Asp f 16 (AF062651), Asp f 17 (AJ224865), Asp f 18 (Y13338), Asp f 22 (AF284645), Asp f 27 (AJ937743), Asp f 28 (AJ937744), Asp f 29 (AJ937745), Asp f 34 (AM496018), Asp n 14 (AF108944) Asp n 25 (L20567), Asp o 21 (M33218). Briefly, each allergen was blotted onto a nitrocellulose membrane in duplicate with PBS and bovine serum albumin (BSA) as controls. The membranes were incubated with 0.1% PBS-Tween-20 followed by plasma in 1:8 dilution with PBS. After 16-hours, membranes were washed on three separate occasions with 0.05% PBS-

Tween-20, first for 15-minutes, followed by 7-minutes (twice) subsequently. After the washing steps, anti-human IgE antibody conjugated with alkaline phosphatase was added and incubated for 2h. Membranes were subsequently analyzed using Syngene imaging software with inter and intra-assay reproducibility above 90%. A sensitisation response was defined as a specific IgE binding intensity above the 95th percentile of the non-diseased control group for each respective allergen.

Anti-Aspergillus IgG: Platella anti-*Aspergillus* IgG (Bio-rad #62783) was performed according to manufacturer's instructions. Ten microliters of serum (in duplicate) were used for assays. Samples with concentrations of >5UA/ml were considered positive for the presence of IgG antibody to *Aspergillus.*

References

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3. Mac Aogain M, Chandrasekaran R, Lim AYH, Low TB, Tan GL, Hassan T, Ong TH, Hui Qi Ng A, Bertrand D, Koh JY, Pang SL, Lee ZY, Gwee XW, Martinus C, Sio YY, Matta SA, Chew FT, Keir HR, Connolly JE, Abisheganaden JA, Koh MS, Nagarajan N, Chalmers JD, Chotirmall SH. Immunological corollary of the pulmonary mycobiome in bronchiectasis: the CAMEB study. *Eur Respir J* 2018: 52(1).

Figure E1: Scatter box plots illustrating differences in (a) Body mass index (BMI) and (b) mMRC dyspnea score between clusters. ns: non-significant, ***p≤0.001. mMRC: Modified Medical Research Council.

Figure E1

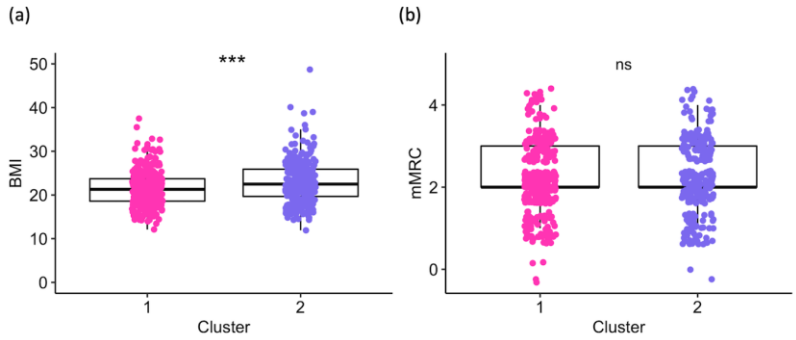

Figure E2: Scattered box plots illustrating exacerbation frequency in relation to COPD gold stage (FEV_1 group) within each cluster. FEV_1: Forced Expiratory Volume in the 1st second. * p≤0.05, **p≤0.01