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Supplementary appendix

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Appendix

Title:

Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose

Authors:

Constanze Kuhlmann, Carla Konstanze Mayer, Mathilda Claassen, Tongai Maponga, Wendy A. Burgers, Roanne Keeton, Catherine Riou, Andrew D. Sutherland, Tasnim Suliman, Megan L. Shaw, Wolfgang Preiser

Corresponding author

Wolfgang Preiser
Division of Medical Virology
Faculty of Medicine and Health Sciences
University of Stellenbosch
National Health Laboratory Service (NHLS) Tygerberg
Tygerberg Campus, Cape Town, South Africa
+27 21 938 9353
preiser@sun.ac.za

Author statements:

The bodies who contributed funding for this study, namely the South African Medical Research Council, Poliomyelitis Research Foundation and National Health Laboratory Service Research Trust, had no role in its conception, conduct or the writing of the Correspondence.

Contributions:

Constanze Kuhlmann (CK) and Carla Konstanze Mayer (CM) (joint 1st authors) and Wolfgang Preiser (WP) (senior author) conceptualised and designed the study, obtained ethics approval and informed consent from the participants and organised logistics. CK and CM acquired clinical data and samples and analysed clinical data. WP, Megan Shaw (MS), Wendy Burgers (WB) and Catherine Riou (CR) oversaw and obtained funding for the laboratory investigations which were conducted by Tongai Maponga (TM), Mathilda Claassen (MC), Andrew Sutherland (AS), Tasnim Suliman (TS) and Roanne Keeton (RK). All authors verified data and analysed results. CK, CM and WP drafted and wrote the manuscript which was reviewed by all authors. All authors had full access to the data and approved submission of this Article. WP, CK and CM had the final responsibility to submit for publication.

Declaration of interests:

We declare no competing interests.

Additional information on sample handling (with references)

Nasopharyngeal swab samples, obtained as dry swabs and stored at +4°C, were eluted in 1.5 millilitre (mL) of phosphate-buffered saline and RNA extracted using the NucliSens easyMAG system (bioMérieux SA, Marcy l'Etoile, France). Viral genome sequences were determined by Oxford Nanopore Technologies (Oxford, UK) sequencing on the GridION using the ARTIC V3 primers set (Engelbrecht et al., 2021) and SARS-CoV-2 RNA loads by quantitative real-time PCR using the E-gene target only (Corman et al., 2020). The *in vitro* transcribed RNA generated with the TranscriptAid T7 High Yield Transcription kit (Thermo Scientific, MA, USA) of a cloned E-gene (courtesy of J. Bhiman, NICD) was used as quantification standard. Serum samples were tested by the Quant II IgG anti-Spike 2-CoV-SARS (Abbott, Illinois, USA) to determine SARS-CoV-2 anti-spike IgG levels (Grupel et al., 2021). To assess T-cell responses, peripheral blood mononuclear cells were isolated from blood samples taken 15-17 days after symptom onset from six of the individuals. After stimulation with SARS-CoV-2 peptide pools (1 µg/mL PepTivator Spike, Nucleocapsid and Membrane, Miltenyi Biotec, Auburn, CA, USA), intracellular cytokine staining and flow cytometry were performed as described (Keeton et al., 2021).

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Participant		Vaccination history						Illness and laboratory findings			
		1st dose		2nd dose		3rd dose		Date of		SARS-CoV-2 viral load	Anti-SARS-CoV-2 spike antibodies
Age	Sex	Date	Vaccine	Date	Vaccine	Date	Vaccine	onset of symptoms	sample	PANGO lineage, GISAID accession ID	
26	f	7 Feb 2021	BNT162b2	28 Feb 2021	BNT162b2	10 Nov 2021	BNT162b2	2 Dec 2021	4 Dec 2021	4.57 log_{10} copies / mL B.1.1.529, EPI_ISL_8152813	15011 AU / mL
27	f	30 Dec 2020	BNT162b2	19 Jan 2021	BNT162b2	3 Oct 2021	CX-024414 (100 µg)	1 Dec 2021	4 Dec 2021	8.22 log_{10} copies / mL B.1.1.529, EPI_ISL_7452792	> 40000 AU / mL
39	m	28 Apr 2021	BNT162b2	13 May 2021	BNT162b2	8 Nov 2021	BNT162b2	2 Dec 2021	5 Dec 2021	4.07 log_{10} copies / mL B.1.1.529, EPI_ISL_7452799	23026 AU / mL
25	f	21 Jan 2021	BNT162b2	11 Feb 2021	BNT162b2	26 Oct 2021	BNT162b2	30 Nov 2021	4 Dec 2021	5.67 log_{10} copies / mL B.1.1.529, EPI_ISL_7452795	19123 AU / mL
25	f	26 Mar 2021	BNT162b2	7 May 2021	BNT162b2	3 Nov 2021	BNT162b2	1 Dec 2021	4 Dec 2021	7.98 log_{10} copies / mL B.1.1.529, EPI_ISL_7452796	18507 AU / mL
25	m	30 Apr 2021	BNT162b2	11 Jun 2021	BNT162b2	3 Nov 2021	BNT162b2	2 Dec 2021	4 Dec 2021	7.37 log_{10} copies / mL B.1.1.529, EPI_ISL_7452797	16752 AU / mL
27	f	28 Feb 2021	ChAdOx1-S	3 May 2021	BNT162b2	26 Oct 2021	BNT162b2	30 Nov 2021	5 Dec 2021	6.77 log_{10} copies / mL B.1.1.529, EPI_ISL_8152814	n.d. (no sample available)

Table 1. Basic demographic data, vaccination history and laboratory findings. Legend: age: in years; sex: f = female, m = male; vaccine: BNT162b2 (Comirnaty; BioNTech, Mainz, Germany); CX-024414 (Spikevax; Moderna, Cambridge, MA, USA); ChAdOx1-S (Vaxzevria; AstraZeneca, Oxford, UK); AU = arbitrary unit; copies / mL = viral RNA copies / mL sample.

	Number of cases (n)						
Clinical symptoms	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Anosmia	0	1	0	0	0	0	0
Backpain	0	1	1	0	0	0	0
Chest pain	0	1	1	1	1	0	0
Chest pressure	2	1	1	1	1	1	1
Conjunctivitis	0	0	0	0	0	0	0
Diarrhoea	0	0	0	0	0	0	0
Dry cough	3	6	7	7	4	4	3
Dysgeusia	0	1	0	0	0	0	0
Fatigue	5	4	4	4	4	5	5
Fever	1	1	1	1	0	0	0
Headache	4	4	2	3	2	0	0
Myalgia	0	2	1	2	1	0	0
Nausea	2	0	0	0	0	0	0
Night sweat	1	1	0	0	0	0	0
Rhinitis	2	4	4	5	3	2	2
Shortness of breath	0	2	3	3	0	0	0
Sinus pressure	2	6	5	4	2	0	0
Skin rash	0	0	1	1	1	0	0
Sore throat	6	6	6	4	2	1	1

Table 2. Clinical symptoms during the observation period. Day 1 = day of onset of symptoms, day 10 = end of isolation period, Day 21 = end of observation period.

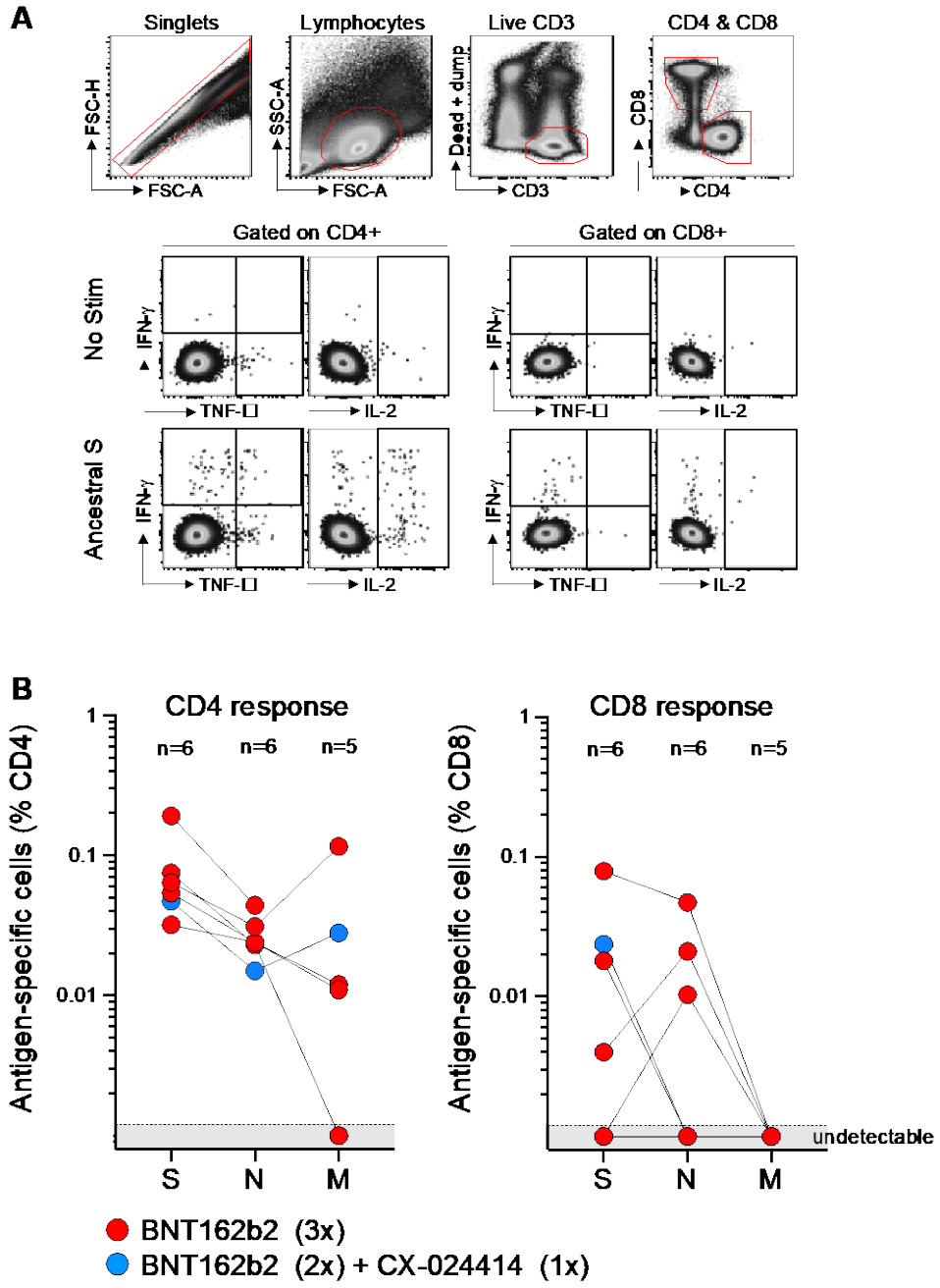

Figure 1. Specific T-cell responses >2 weeks after Omicron breakthrough infection. A. Gating strategy and representative example of intracellular staining showing the production of IFN-g, TNF-a and IL-2 cytokines in response to ancestral SARS-CoV-2 Spike peptide pool. **B.** Frequencies of CD4+ T cells (left panel) and CD8+ T cell (right panel) producing any of the measured cytokines (IFN-g, TNF-a or IL-2) in response to ancestral Spike (S), Nucleocapsid (N) and Membrane (M) peptide pools.

Legend: BNT162b2 (Comirnaty, BioNTech, Mainz, Germany); CX-024414 (Moderna, Cambridge, MA, USA).