

## Correspondence on 'SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response'

We read with a great interest the article published by Bonelli *et al* suggesting an inducible cellular immune response in rituximab (Rtx) treated patients.<sup>1</sup> The CD20-antibody Rtx is one of the most widespread biologicals worldwide with a broad spectrum of oncological and rheumatological indications. Due to its depleting effect on circulating B cells, the generation of antibodies against novel pathogens is impaired in Rtx-treated patients.<sup>2,3</sup> Accordingly, the last EULAR recommendations on vaccination advised that 'vaccination should be provided at least 6 months after the last administration and 4 weeks before the next course of B cell-depleting therapy'.<sup>4</sup> To ensure appropriate SARS-CoV-2 vaccination, the last EULAR advise was to refer to a rheumatologist.<sup>5</sup> The American College of Rheumatology (ACR) has recommended to vaccinate Rtx-treated patients not earlier than 5 months after the last administration with the next cycle given not earlier than 2–4 weeks thereafter.<sup>6</sup> In any case, the combination of B cell-depleting therapy with vaccination has been quite a challenge for patients and physicians—especially since it became clear that Rtx therapy may be associated with unfavourable outcomes in B cell-depleted patients.<sup>7</sup> Fortunately, very recent data by Bonelli *et al* have now suggested that a cellular response is mounted after SARS-CoV-2 vaccination in Rtx-treated patients despite a failed humoral immune response.<sup>1</sup> The authors demonstrated that peripheral blood cells of vaccinated patients do produce Interferon  $\gamma$  (IFN $\gamma$ ) after stimulation with SARS-CoV-2 spike (S) protein-derived overlapping peptides.<sup>1</sup> These results increase the scientific interest into a more detailed characterisation of vaccine-reactive T-cell immunity, which has recently been in the focus of our group as well due to a frequent Rtx application in our settings.

Applying multiparameter flow cytometry, we explored the efficacy of SARS-CoV-2 vaccination as defined by quantification and in-depth characterisation of T cell immunity in nine Rtx-treated patients diagnosed with autoantibodies against myeloperoxidase (MPO), proteinase 3 (PR3), MPO/PR overlap and immunoglobulin A (IgA) vasculitis or membranous glomerulopathy (table 1). Their mean age was 65 years, 33.3% were women, and the mean time after the last application of Rtx was 4.5 months (2–7 months). The vaccination by two doses of BNT162b2 was performed within 3 weeks. SARS-CoV-2-reactive immunity was

analysed before the first dose, and 3 weeks after the first and the second dose, respectively. Vaccinated healthcare workers (n=14) served as controls.

All but two Rtx-treated patients had neglectable levels of CD19<sup>+</sup> B cells (figure 1D). Consequently, the development of antibodies to the SARS-CoV-2 S-protein was substantially impaired in Rtx group excluding two of the patients with detectable CD19<sup>+</sup> B cells, who showed seroconversion. In contrast, virus specific IgG antibodies were detected in all healthy controls.

Importantly, vaccine-reactive T cells were found in the majority of Rtx-treated patients. Due to pre-existing SARS-CoV-2-cross-reactive T cells known to be detectable in unexposed patients as demonstrated by other and our groups,<sup>8,9</sup> vaccine-directed T cell response was defined by a >twofold increase of SARS-CoV-2 S-protein-reactive T cell frequencies compared with the prevaccination (TP0) state. Accordingly, CD4<sup>+</sup> T cell vaccination response was observed in 78% of Rtx-treated patients 3 weeks after the first vaccination and 86% after the second vaccination (figure 1A), while CD8<sup>+</sup> T cell responses were only found in 22% and 43% of the patients after the first and second vaccinations, respectively. Of note, there were no statistically significant differences in the frequencies of vaccine-reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells between patients and controls (figure 1B). The substantial number of activated T cells produced GranzymeB, interleukin (IL)-2, interferon  $\gamma$  (IFN $\gamma$ ) or tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) as monofunctional or polyfunctional T cells suggesting their protective function<sup>10</sup> (figure 1C). Again, there were no statistically significant differences between patients and controls.

T cell reactivity against SARS-CoV-2 variants of concern (VOC), including B.1.1.7 and B.1.351 strains, after vaccination with the Wuhan wild type S-protein are of special interest. Importantly, 75% of Rtx-treated patients had T cells directed against the S-protein derived from both mutant strains after the second vaccination, respectively (figure 1B). These T cells were able to produce several cytokines simultaneously suggesting antiviral potential of these polyfunctional T cells<sup>10</sup> (figure 1C). Again, the magnitude and functionality of B.1.1.7 and B.1.351 S-reactive T cells were not significantly different between patients and controls (figure 1B), with a tendency towards lower frequencies in the former.

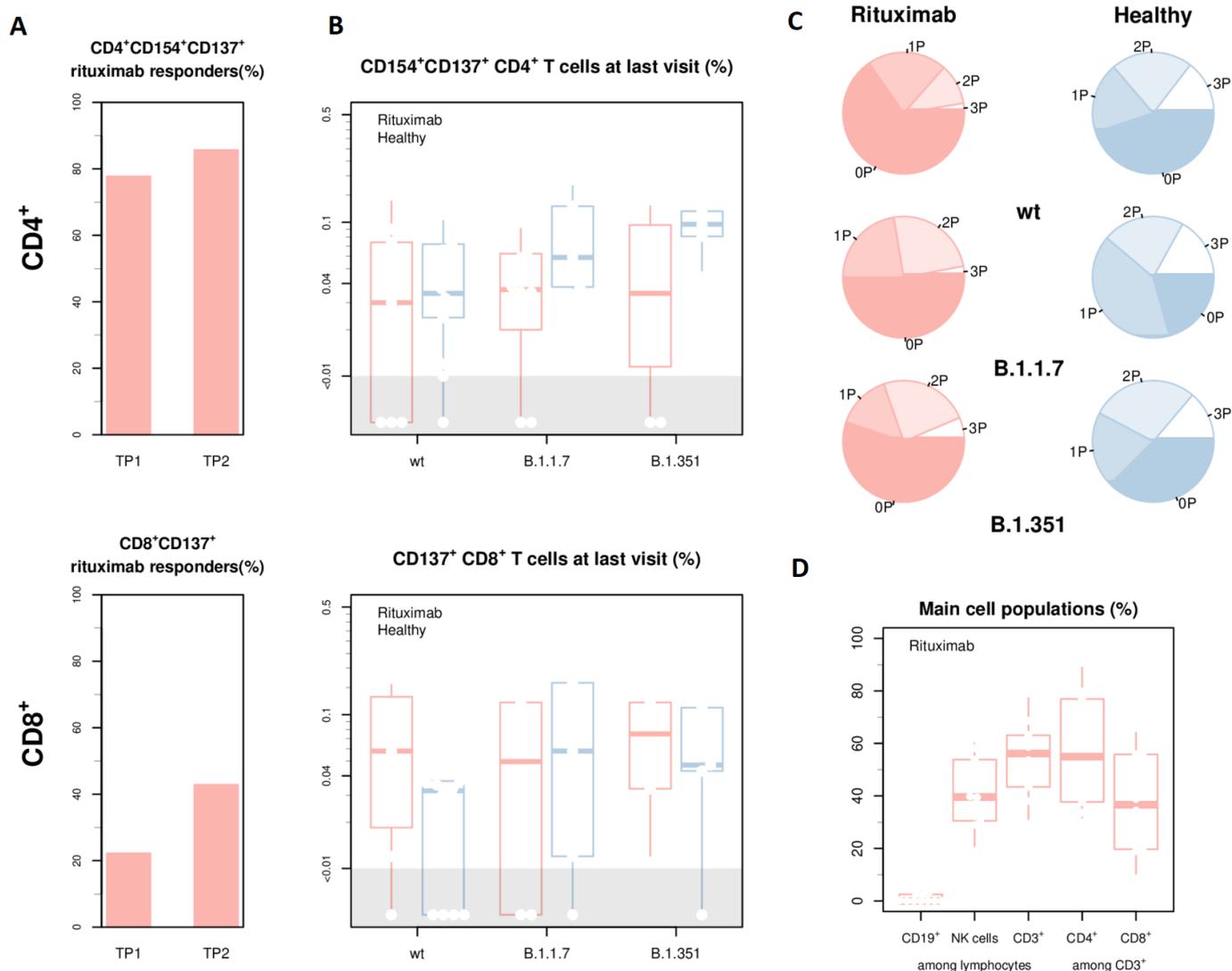
In conclusion, despite the lack of seroconversion in most patients, Rtx-treated patients are able to raise T cells reactive not only to SARS-CoV-2 wild type strain but also to B.1.1.7 and B.1.351 VOC. Although not a confirmation of antiviral protection of vaccine-reactive T cells, their polyfunctional properties suggest an antiviral potential.

**Table 1** Patient characteristics

	Age	Sex	Body mass index (kg/m <sup>2</sup> )	Months since rituximab	Overall rituximab treatment duration	Aetiology	GFR (CKD-EPI; mL/min/1.73 <sup>2</sup> )
1	73	F	21.5	2	8	MPO	31.7
2	65	M	32.2	2	26	PR3	38.6
3	60	M	35.4	7	26	IgAV	17.3
4	57	M	23.3	6	6	MPO	47.1
5	74	M	32.1	7	7	MGN	45.2
6	79	M	25.1	5	27	MGN	43.7
7	52	F	25.7	4	17	MPO/PR3	43.2
8	64	M	27.5	4	21	PR3	27.5
9	64	F	34.6	4	10	PR3	47.9

GFR, glomerular filtration rate

; IgAV, IgA-associated vasculitis; MGN, membranous glomerulonephritis; MPO, myeloperoxidase; PR3, proteinase 3.



**Figure 1** Robust polyfunctional T cell response directed against spike (S) wild type (wt) and variants of concern (VOC) strains can be detected in rituximab (Rtx) treated patients following SARS-CoV-2 vaccination. (A) Incidence of Rtx-treated patients responded to the vaccination 3 weeks after the first dose (TP1) and 3 weeks after the second dose (TP2). Vaccine-directed T cell response was defined as >twofold increase of S-reactive T cell frequencies as compared with the prevaccination (TP0) T cell response (CD4<sup>+</sup> T cells top; CD8<sup>+</sup> T cells bottom). (B) The relative frequency of SARS-CoV-2 reactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells directed against S-protein of wt, B.1.1.7 and B.1.351 VOC strains in Rtx-treated patients and healthy donors. For CD4<sup>+</sup> T cells, activation is defined by CD154<sup>+</sup> and CD137<sup>+</sup> double expression (top) and for CD8<sup>+</sup> T cells by CD137<sup>+</sup> (bottom). Presented are data obtained from the last visit (3–5 weeks) after the second vaccination dose. For wt strain, 9 Rtx-treated patients and 14 controls were available. For VOC strains, eight Rtx-treated patients and five controls were available. PBCMs were isolated 3–5 weeks after the second BNT162b2 dose and stimulated overnight with SARS-CoV-2 S-protein of wt, B.1.1.7 and B.1.351 strains, respectively. T cell reactivity was determined by flow cytometry as CD154<sup>+</sup>, CD137<sup>+</sup> and CD137<sup>+</sup> for CD4<sup>+</sup> and CD8<sup>+</sup>, respectively, together with antibodies for Granzyme B, IFN $\gamma$ , TNF $\alpha$  and IL-2. (C) Total frequency of monofunctional (1P), bifunctional (2P) or trifunctional (3P) T cells concurrently producing one, two or three cytokines or no cytokines (0P), respectively, in response to S-protein from wt, B.1.1.7 or B.1.351. PBCMs were isolated 3–5 weeks after the second BNT162b2 dose and stimulated overnight with SARS-CoV-2 S-protein from wt, B.1.1.7 and B.1.351. T cell reactivity was determined by flow cytometry as CD154<sup>+</sup> and CD137<sup>+</sup> together with antibodies for Granzyme B, IFN $\gamma$ , TNF $\alpha$  and IL-2. (D) Reduced CD19<sup>+</sup> but normal frequencies of other lymphocytes in Rtx-treated patients. The frequencies of CD19<sup>+</sup>, NK, CD3<sup>+</sup>, CD4<sup>+</sup>/CD3<sup>+</sup> and CD8<sup>+</sup>/CD3<sup>+</sup> were evaluated in fresh whole blood by flow cytometry. Each point signifies a patient. IFN $\gamma$ , Interferon  $\gamma$ ; IL, interleukin, NK, natural killer, TNF $\alpha$ , tumour necrosis factor  $\alpha$ .

The role of SARS-CoV-2-specific antibodies in COVID-19 is not well established: reports indicate an increased risk of severe COVID-19 infections in Rtx-treated patients,<sup>7</sup> whereas other data have suggested that B cells are dispensable for resolving such infections.<sup>11</sup> The here presented data indicate that polyfunctional antiviral T cell responses are raised after SARS-CoV-2 vaccination in this high-risk population suggesting protection in the absence of virus-specific antibodies.

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**Acknowledgements** We thank the technical personnel of Immune Diagnostic Laboratory, Marien Hospital Herne, for the excellent technical support during the study.

**Contributors** TW, FSS, AD, SD, KP, BW, BH and JB were responsible for the patient management, clinical data collection, sample collection and immunisation. MA, SS and US were involved in sample analysis. AB-N was responsible for statistical analysis and graphical presentation. NB, TW, FSS and US prepared and revised draft of the manuscript. NB supervised the study. All authors have read and revised manuscript, if required.

**Funding** The study was supported by grant of Mercator Stiftung and BMBF grant noChro.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

**Patient consent for publication** Not required.

**Ethics approval** The study was approved by the local ethical committee (Ruhr University Bochum, application number 20–7126). All study participants provided a written informed consent.

**Provenance and peer review** Not commissioned; internally peer reviewed.

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**To cite** Westhoff TH, Seibert FS, Anft M, et al. *Ann Rheum Dis* Epub ahead of print: [please include Day Month Year]. doi: 10.1136/annrheumdis-2021-220756

Received 10 May 2021  
Accepted 11 May 2021



► <http://dx.doi.org/10.1136/annrheumdis-2021-220764>

*Ann Rheum Dis* 2021;0:1–3. doi:10.1136/annrheumdis-2021-220756

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#### REFERENCES

- Bonelli MM, Mrak D, Perkmann T, et al. SARS-CoV-2 vaccination in rituximab-treated patients: evidence for impaired humoral but inducible cellular immune response. *Ann Rheum Dis* 2021;annrheumdis-2021-220408.
- van der Kolk LE, Baars JW, Prins MH, et al. Rituximab treatment results in impaired secondary humoral immune responsiveness. *Blood* 2002;100:2257–9.
- Deepak P, Kim W, Paley MA, et al. Glucocorticoids and B cell depleting agents substantially impair immunogenicity of mRNA vaccines to SARS-CoV-2. *medRxiv* 2021. doi:10.1101/2021.04.05.21254656. [Epub ahead of print: 09 Apr 2021].
- Furer V, Rondaan C, Heijstek MW, et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. *Ann Rheum Dis* 2020;79:39–52.
- Bugatti S, Balduzzi S, De Stefano L, et al. Correspondence on 'EULAR December 2020 viewpoints on SARS-CoV-2 vaccination in patients with RMDs'. *Ann Rheum Dis* 2021;80:411–2.
- Curtis JR, Johnson SR, Anthony DD, et al. American College of Rheumatology Guidance for COVID-19 Vaccination in Patients with Rheumatic and Musculoskeletal Diseases - Version 1. *Arthritis Rheumatol* 2021. doi:10.1002/art.41734. [Epub ahead of print: 17 Mar 2021].
- Avouac J, Drumez E, Hachulla E. FAI2R/SFR/SNFM/SOFREMIP/CRI/IMIDIATE Consortium and contributors. COVID-19 outcomes in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. *Lancet Rheumatol* 2021.
- Thieme CJ, Anft M, Paniskaki K, et al. Robust T cell response toward spike, membrane, and nucleocapsid SARS-CoV-2 proteins is not associated with recovery in critical COVID-19 patients. *Cell Rep Med* 2020;1:100092.
- Braun J, Loyal L, Frensch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020;587:270–4.
- Betts MR, Nason MC, West SM, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 2006;107:4781–9.
- Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell* 2021;184:861–80.