Cytokine release syndrome in a patient with colorectal cancer after vaccination with BNT162b2

Lewis Au1,2,15, Annika Fendler1,15, Scott T. C. Shepherd1,2, Karolina Rzeniewicz1, Maddalena Cerrone3,4, Fiona Byrne1, Eleanor Carlyle2, Kim Edmonds2, Lyra Del Rosario2, John Shon6, Winston A. Haynes9, Barry Ward1, Ben Shum1,2, William Gordon1, Camille L. Gerard1,6, Wenyi Xie1, Nalinie Joharatnam-Hogan2, Kate Young2, Lisa Pickering2, Andrew J. S. Furness2, James Larkin2, Ruth Harvey7, George Kassiotis8, Sonia Gandhi9,10, Crick COVID-19 Consortium, Charles Swanton11, Charlotte Fribbens12,13, Katalin A. Wilkinson3, Robert J. Wilkinson3,4, David K. Lau13, Susana Banerjee14, Naureen Starling13, Ian Chau15,15, CAPTURE Consortium* and Samra Turajlic8,12,15

Patients with cancer are currently prioritized in coronavirus disease 2019 (COVID-19) vaccination programs globally, which includes administration of mRNA vaccines. Cytokine release syndrome (CRS) has not been reported with mRNA vaccines and is an extremely rare immune-related adverse event of immune checkpoint inhibitors. We present a case of CRS that occurred 5 d after vaccination with BTN162b2 (tozinameran)—the Pfizer-BioNTech mRNA COVID-19 vaccine—in a patient with colorectal cancer on long-standing anti-PD-1 monotherapy. The CRS was evidenced by raised inflammatory markers, thrombocytopenia, elevated cytokine levels (IFN-γ, IL-2R/IL-18/IL-16/IL-10) and steroid responsiveness. The close temporal association of vaccination and diagnosis of CRS in this case suggests that CRS was a vaccine-related adverse event; with anti-PD1 blockade as a potential contributor. Overall, further prospective pharmacovigilence data are needed in patients with cancer, but the benefit-risk profile remains strongly in favor of COVID-19 vaccination in this population.

CRS/cytokine storm is a systemic inflammatory response, characterized by excessive cytokine release (that is, elevated INF-γ, IL-6, IL-10 and IL-2R)1. CRS might develop after infection (including COVID-19) or due to iatrogenic causes, most notably chimeric antigen receptor T cell (CAR-T) therapy and, less frequently, cytotoxic chemotherapy or stem cell transplantation1,2. Extremely rarely, it occurs after immune checkpoint inhibitor (ICI) therapy3,4, and, to our knowledge, it has not been reported after administration of any vaccine. Here we report a case of CRS after vaccination with BNT162b2 (tozinameran), an mRNA COVID-19 vaccine.

A 58-year-old male commenced anti-PD-1 monotherapy (an investigational ICI within an ongoing interventional clinical trial; NCT02715284) in February 2019 for the treatment of mismatch repair-deficient colorectal cancer (MMRd CRC) metastatic to mesentry and rectus muscle (Fig. 1a). Two months after treatment initiation, he experienced a neurological immune-related adverse event (irAE) with worsening ataxia (grade 1 to grade 2, and magnetic resonance imaging changes in pons, medulla and cerebellum) on the background of pre-existing spinocerebellar ataxia of unknown etiology. ICI was suspended, and he was commenced on 1 mg kg−1 prednisolone (tapered over 1 month), and ataxia returned to grade 1 (baseline). Anti-PD-1 therapy was re-started in June 2019 (Fig. 1a), with stable disease as per immune-related Response Evaluation Criteria in Solid Tumors. In March 2020 (13 months after commencing ICI), he developed an endocrine irAE (grade 1 hypocortisolemia from adrenocorticotropic hormone deficiency; Fig. 1a) and was commenced on physiological corticosteroid replacement (prednisolone, 3 mg daily). Disease control was maintained, and the last ICI dose was administered in December 2020, 27 d before BNT162b2.

The patient had no history of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and had negative SARS-CoV-2 serological tests in June and October 2020. He received the first dose of BNT162b2 vaccine on 29 December 2020 (Fig. 1a) without immediate adverse events, except for grade 1 inflammation at the vaccination site. Five days later (32 d after the last anti-PD-1 dose), he presented with myalgia, 2-d history of diarrhea (grade 1) and 1-d history of fever (38.4 °C) despite anti-pyretics (ibuprofen) use. On admission to the hospital, his vital signs were as follows: oxygen saturation, 100% on room air; respiratory rate, 18 breaths per minute; blood pressure, 111/71 mmHg; heart rate, 86 beats per minute; and temperature, 36.7°C. Laboratory investigations revealed elevated inflammatory markers (C-reactive protein (CRP)),...
125 mg L$^{-1}$ (normal, <6 mg L$^{-1}$); serum lactate dehydrogenase (LDH), 184 U L$^{-1}$ (normal range, 120–246 U L$^{-1}$); and thrombocytopenia ($68 \times 10^9$ cells per liter (normal range, 150–410 cells per liter)), confirmed on microscopy (Fig. 1b). Empirical treatment with broad-spectrum intravenous antibiotics was commenced; however, blood and urine cultures were negative, as was SARS-CoV-2 RT–PCR of serial nasopharyngeal swabs (Fig. 1a). There were no clinical signs or symptoms during admission or follow-up to suspect a thrombotic event in this patient. Computed tomography of thorax, abdomen and pelvis revealed no nidus of infection or thrombosis in this case. Most cytokines decreased substantially during IVMP treatment, but persistent elevation of IL-2R, IL-2, IL-16 and IL-18 in this case. Most cytokines decreased substantially during IVMP treatment, but persistent elevation of IL-2R, IL-2, IL-16 and IL-18 on day 12 of admission (Fig. 2a) indicated sustained T cell activation. SI-reactive and neutralizing antibodies were detectable 7 d after vaccination (Fig. 2b), and the titers continued to rise during IVMP treatment, suggesting a robust vaccine-induced immune response. However, S-specific CD4$^+$ and CD8$^+$ T cells were undetectable on days 17 and 40 after vaccination, and no IFN-γ-producing T cells were detected (Fig. 2c,d,e and Extended Data Fig. 1a,b), consistent with reports in patients without cancer after the first dose of BNT162b2 (ref. 5). Data on the effect of steroids on mRNA vaccine-specific immune response. One study demonstrated that dexamethasone given before, but not after, an mRNA-based cancer vaccine resulted in reduced activation of antigen-specific T cells$^6$. In the case under study, steroids were administered 10 d after vaccination and unlikely to have affected vaccine-specific immune response.

To identify antibody-binding epitopes, we performed a serum epitope repertoire analysis (SERA) and a protein-based immunome-wide association study (PIWAS), using a bacterial display system coupled with next-generation sequencing.
The post-vaccine profile was similar to that of healthcare workers after COVID-19 mRNA vaccine, with a rise in positive signal against spike but not non-spike proteins (versus patients positive for SARS-CoV-2) (Extended Data Fig. 2a,b). This was consistent with the lack of prior SARS-CoV-2 infection as a potential contributor to the clinical presentation.

Isolated thrombocytopenia (without thrombosis) has been described with mRNA-based COVID-19 vaccines, including BNT162b2 (ref. 8). Given recent reports of a pathogenic platelet factor-4 (PF4)-dependent syndrome leading to thrombotic thrombocytopenia after vaccination with ChAdOx1 nCoV-19 (AstraZeneca)9–11, which is a viral vector-based COVID-19 vaccine, we evaluated for PF4 antibodies, which were not detectable (Extended Data Fig. 3). Although this result does not comprehensively exclude an independent mechanism for the observed thrombocytopenia, the constellation of clinical and laboratory findings make thrombocytopenia likely to be a component of CRS in this case.

Fig. 2 | Cytokine profile and immune response to BNT162b2 vaccine. a, Cyto/chemokine levels were measured using the human immune monitoring 65-plex ProcartaPlex immunoassay in consecutive plasma samples. Samples were measured in duplicates. Data are presented as the log10 of the concentration in pg ml⁻¹. b, Kinetics of S1-reactive and neutralizing antibody responses after BNT162b2. Data are presented as the reciprocal dilution of the last detected sample. IVMP treatment is indicated in red. c, SARS-CoV-2-specific CD4⁺ and CD8⁺ T cell response in exemplary samples after stimulation of PBMCs with spike (S) peptide pool. d, PBMCs were stimulated with S, S1 and S+ peptide pools representing the full length of the spike protein. SEB was used as a positive control. Data are presented as a stimulation index indicating the ratio of the frequency of CD4⁺CD137⁺OX40⁺ or CD8⁺CD137⁺CD69⁺ T cells in the sample and the negative control. SEB, Staphylococcal enterotoxin B.
The laboratory findings in CRS are variable and relate to the underlying cause, although CRP elevation is characteristic and correlates with severity. Elevated ferritin and thrombocytopenia are also common abnormalities. Although there are no defined cytokine profiles that confirm CRS, raised IFN-γ, IL-2R, IL-18, IL-6 and IL-10 are considered key in establishing the diagnosis. All except IL-6 were elevated in this case. Although transient cytokine elevation (IFN-α, IFN-γ, IL-6, IFN-inducible protein-10 and IL-12p70) was observed after mRNA cancer vaccines co-administered with ICI in patients with melanoma, they manifest as self-limiting mild flu-like symptoms.

Fewer than 0.01% of irAEs reported in the context of anti-PD-1 monotherapy involve CRS, and, to date, no CRS events have been reported after either BNT162b2 or the mRNA-1273 vaccine (Moderna)—the two mRNA-based COVID-19 vaccines currently available. ICI-related CRS typically develops a median of 4 weeks after ICI initiation (range, 1–18 weeks), making ICI as the sole cause of CRS unlikely in this patient, who commenced anti-PD1 treatment 22 months prior. The close temporal association of vaccination and clinical presentation favors the vaccine as the potential trigger of CRS in this case.

Receptor occupancy associated with anti-PD-1 agents is 2–3 months, and it remains possible that CRS was triggered by the vaccine on a background of immune activation secondary to PD1 blockade that results in T cell proliferation and increased effector function. We did not detect S-reactive T cells in the periphery, and a direct mechanism for T cells driving CRS in this case could not be demonstrated. However, vaccine-activated T cells that contributed to CRS could be resident within tissue or lymph nodes and, therefore, undetectable in the blood. T cell cross-reactivity, as a result of sequence similarity between spike protein and tumor neoantigens, is an alternative, although less likely, cause of CRS in this case. Cross-reactivity to cardiac tissue was reported as a mechanism of ICI-related myocarditis, and this patient’s history of irAEs and the high neoantigen load (typical of MMRd CRC) could, in theory, increase the likelihood of T cell cross-reactivity.

Given that patients with cancer were excluded from SARS-CoV-2 vaccine studies and are currently prioritized in COVID-19 vaccination programs globally, this case motivates prospective pharmacovigilance regarding the safety profile of COVID-19 vaccines in patients with cancer. So far, prospective data have not demonstrated additional safety concerns of BNT162b2 administration either in patients with cancer generally (n = 1519) or, specifically, in those who have been treated with ICI (n = 170). Current empirical recommendations regarding the timing of COVID-19 vaccination suggest administering ‘on availability’ in patients with cancer on systemic anti-cancer treatments, including ICI, cytotoxic chemotherapy and hormone therapy, and avoiding vaccination within 48–72 h of investigational products to minimize misattribution of adverse event causation.

Overall, as CRS in this case is an isolated report, and patients with cancer are generally more vulnerable to COVID-19 (refs. 22,23), the benefit–risk profile for COVID-19 vaccination remains strongly in favor of vaccination in this population. It is of critical importance that patients with cancer remain prioritized during vaccine rollout.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01387-6.

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Crick COVID-19 Consortium

George Kassiotis\textsuperscript{8}, Sonia Gandhi\textsuperscript{9,10} and Charles Swanton\textsuperscript{11}

A full list of members appears in the Supplementary Information.

CAPTURE Consortium

Lewis Au\textsuperscript{1,2}, Annika Fendler\textsuperscript{1}, Scott T. C. Shepherd\textsuperscript{1,2}, Fiona Byrne\textsuperscript{1}, Ben Shum\textsuperscript{1,2}, Camille Gerard\textsuperscript{1,6}, Kate Young\textsuperscript{2}, Lisa Pickering\textsuperscript{2}, Andrew J. S. Furness\textsuperscript{2}, James Larkin\textsuperscript{2}, George Kassiotis\textsuperscript{8}, Katalin A. Wilkinson\textsuperscript{3}, Robert J. Wilkinson\textsuperscript{3,4}, Susana Banerjee\textsuperscript{14}, Naureen Starling\textsuperscript{13}, Ian Chau\textsuperscript{13} and Samra Turajlic\textsuperscript{1,2}

A full list of members appears in the Supplementary Information.
Methods

CAPTURE design, study schedule and follow-up. During admission, the patient was enrolled in CAPTURE (NCT03226886; see Supplementary Material for a list of consortium members), an observational prospective study of the immune response to SARS-CoV-2 in patients with cancer that opened for recruitment in May 2020 at the Royal Marsden NHS Foundation Trust. The study design was previously published24. Adult patients with current or history of invasive cancer were enrolled in CAPTURE (NCT03226886). TRACERx Renal was initially approved by the National Research Ethics Service (NRES) Committee London - Fulham on 17 January 2012. The TRACERx Renal sub-study CAPTURE was submitted as part of Substantial Amendment 9 and approved by the Health Research Authority on 30 April 2020 and the NRES Committee London - Fulham on 1 May 2020. The CAPTURE protocol was approved by institutional review boards and ethics committees, and the participant in this case report gave written informed consent for sample collection and use, according to CARE guidelines and in compliance with Declaration of Helsinki principles.

Adverse events grading. All adverse events were graded per Common Terminology Criteria for Adverse Events version 4.03.

Handling of whole blood samples. For indicated experiments, serum or plasma samples were heat inactivated at 56°C for 30 min before use.

Plasma and peripheral blood mononuclear cell isolation. Whole blood was collected in EDTA tubes (VWR) and stored at 4°C until processing. All samples were processed within 24 h. Time of blood draw, processing and freezing was recorded for each sample. Before processing, tubes were brought to room temperature. Peripheral blood mononuclear cells (PBMCs) and plasma were isolated by density gradient centrifugation using pre-filled centrifugation tubes (pluriSelect). Up to 30 ml of undiluted blood was added on top of the sponge and centrifuged for 30 min at 1,000g at room temperature. Plasma was carefully removed and then centrifuged for 10 min at 4,000g to remove debris, aliquoted and stored at −80°C. The cell layer was then collected and washed twice in PBS by centrifugation for 10 min at 300g at room temperature. PBMCs were resuspended in Recovery Cell Culture Freezing Medium (Thermo Fisher Scientific) containing 10% DMOS, placed overnight in Cool Cell freezing containers (Corning) at −80°C and then stored in liquid nitrogen.

Serum isolation. Whole blood was collected in serum coagulation tubes (Vacuette CAT tubes, Greiner Bio-One) for serum isolation and stored at 4°C until processing. All samples were processed within 24 h. Time of blood draw, processing and freezing was recorded for each sample. Before processing, tubes were brought to room temperature. Serum or plasma samples were heat inactivated at 56°C for 4 h at 37°C in 5% CO₂ in duplicates. The inoculum was then removed, and cells were overlaid with viral growth medium. Cells were incubated at 37°C in 5% CO₂. At 24 h after infection, cells were fixed in 4% paraformaldehyde (PFA) and permeabilized with 0.1% Triton X-100/PBS. Virus plaques were visualized by immunostaining, as described previously for the neutralization of influenza viruses using a rabbit polyclonal anti-NSP8 antibody used at 1:1,000 dilution and anti-rabbit horseradish peroxidase (HRP)-conjugated antibody at 1:1,000 dilution and detected by action of HRP on a tetramethyl benzidine-based substrate. Virus plaques were quantified, and ID₅₀ was calculated.

T cell stimulation. PBMCs for in vitro stimulation were thawed at 37°C and resuspended in 10 ml of warm complete medium (RPMI and 5% human AB serum) containing 0.02% benzonase. Viable cells were counted, and 2×10⁶ cells were seeded in 200 µl of complete medium per well of a 96-well plate. Cells were stimulated with 4 µl per well of PepTigov SARS-CoV-2 S, M, or N pools (representing 1 µg ml⁻¹ final concentration per peptide; Miltenyi Biotec). Staphylococcal enterotoxin B (Merck) was used as a positive control at 0.5 µg ml⁻¹ final concentration; negative control was PBS containing DMOS at 0.002% final concentration. PBMCs were cultured for 24 h at 37°C in 5% CO₂.

Activation-induced marker assay. Cells were washed twice in warm PBS. Dead cells were stained with 0.5 µl per well of Zombie dye V500 for 15 min at room temperature in the dark and then washed once with PBS containing 2% FCS (FACS buffer). A surface staining mix was prepared per well, containing 2 µl per well of each antibody for surface staining (Supplementary Table 1) in 50:50 brilliant stain (BD Biosciences) and FACS buffer. PBMCs were stained with 50 µl of surface staining mix per well for 30 min at room temperature in the dark. Cells were washed once in FACS buffer and fixed in 1% PFA in FACS buffer for 20 min and then washed once and resuspended in 200 µl of PBS. All samples were acquired on a Bio-Rad ZE’5 flow cytometer running Bio-Rad Everest software version 2.4 and analyzed using FlowJo version 10 (Tree Star) analysis software. Compensation was performed with 20 µl of antibody-stained Anti-Mouse Ig, k/Negative Control Compensation Particles Set (BD Biosciences). Up to 1x 10⁵ live CD1⁴ CD1⁴ cells were acquired per sample. Gates were drawn relative to the unstained control for each donor. Gating strategy is shown in Supplementary Fig. 2c. T cell response is displayed as a stimulation index by dividing the percentage of apoptosis inhibitor of macrophage (AIM)-positive cells by the percentage of cells in the negative control. When S, M and N stimulation were combined, the percentage of positive cells was divided by the three times the percentage of positive cells in the negative control.

ELISpot assay. IFN-γ pre-coated ELISpot plates (Mabtech) were blocked with complete medium (RPMI and 5% human AB serum) before 300,000 PBMCs were seeded per well and stimulated with 18h with 2 µl per well of PepTigov SARS-CoV-2 S, M or N pools (representing 1 µg ml⁻¹ final concentration per peptide; Miltenyi Biotec). Plates were developed with human biotinylated IFN-γ detection antibody (7-B6-1-ALP, 1:200), followed by incubation with BCIP/NBT Phosphatase Substrate (SeraCare). Spot-forming units (SFU) were quantified with ImmunoSpot (Mabtech). To quantify positive peptide-specific responses, spots of the unstained wells were subtracted from the peptide-stimulated wells, and the results were expressed as SFU/106 PBMCs.

Multiplex immune assay for cytokines and chemokines. The pre-configured multiplex Human Immune Monitoring 65-plex ProcartaPlex immunoassay kit (Invitrogen, Thermo Fisher Scientific) was used to measure 65 protein targets in plasma on the Bio-Plex platform (Bio-Rad Laboratories), using Luminex xMAP technology: Analytes measured included: APRIL; BLEC; CD30; CD40L; ENA- 78; eotaxin; eotaxin-2; fractalkine; G-CSF; GM-CSF; GRO-alpha; HGF; IL-1α; IL-1β; IL-2; IL-5; IL-6; IL-7; IL-8; IL-9; IL-10; IL-12; IFN-γ; IL-18; IL-1ß; IL-16; IL-17; IL-17A; IL-18; IL-1α; IL-1β; IL-2; IL-7; IL-15; IL-16; IL-17F; IL-20; IL-22; IL-23; IL-26; IL-27; IL-28B; IL-31; IL-1α; IL-1β; IL-2; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IP-10; I-TAC; IFN-α; MCP-1; MCP-2; MCP-3; M-CSF; MDC; MIP; MIP-1α; MIP-1β; MIP-3α; MIP-3β; NGF-β; SCF; SDF-1α; TNF-β; TNF-α; TNF-R2; TRAIL; TSLP; TWEAK; and VEGF-A. All assays were conducted as per manufacturer recommendations.

SERA. Patient serum samples were screened and analyzed using the previously published SERA pipeline27. Briefly, sera were screened with a randomized bacterial peptide display library, and plasmids from antibody-bound bacteria were isolated and sequenced. PIWAS was applied to identify epitopes and antigens for the SARS-CoV-2 proteome.

PF-4 IgG assay. Patient serum samples were analyzed using the LIFECODES PF-4 IgG Solid Phase ELISA microwells assay (Immunocor). Briefly, diluted serum and controls were added to microwells coated with PF-4 complexed to polyvinyl sulfate, incubated for 45 min at 37°C and washed. IgG conjugate was added and incubated for 45 min at 37°C. Plates were developed by incubation NPFP solution. Absorbance of each well was read at 405 nm. A positive and negative serum control was measured on the same plate. Values greater than 0.4 were considered positive.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All requests for raw and analyzed data, materials and CAPTURE study protocol will be reviewed by the CAPTURE Trials Team, Skin and Renal Clinical Trials Unit, The Royal Marsden NHS Foundation Trust (CAPTURE@rmh.nhs.uk) to
determine whether the request is subject to confidentiality and data protection obligations. Data and materials that can be shared will be released via a materials transfer agreement.

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Author contributions

S.T., L.A., S.S. and A.F. contributed to study design. L.A., K.R., A.F. and S.T. drafted the manuscript. S.S., A.F. and L.A. contributed to the collection and processing of clinical specimens. A.F., K.W. and M.C. performed laboratory analysis. A.F., L.A. and K.R. contributed to visualizations. S.S., L.A., N.J.H., D.L., I.C., N.S. and C.F. provided clinical data. All authors critically reviewed the manuscript for intellectual content, approved the final version of the manuscript for submission and agreed to be accountable for all aspects of the work.

Competing interests

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C.S. has consulted for Amgen, AstraZeneca, Bicycle Therapeutics, Bristol Myers Squibb, Celgene, Genentech, GlaxoSmithKline, GRAIL, Illumina, Medixce, Merck Sharp & Dohme, Novartis, Pfizer, Roche-Ventana and the Sarah Cannon Research Institute. C.S. has stock options in Apogen Biotechnologies, Epic Biosciences and GRAIL, and has stock options and is co-founder of Achilles Therapeutics. C.S. holds pending patents relating to assay technology to detect tumor recurrence (PCT/GB2017/053289); targeting neoantigens (PCT/E2016/059401); identifying patent response to immune checkpoint blockade (PCT/E2016/071471); determining whether HLA LOH is lost in a tumor (PCT/GB2018/052004); predicting survival rates of patients with cancer (PCT/GB2020/052021); treating cancer by targeting insertion/deletion mutations (PCT/GB2018/051893); identifying insertion/deletion mutation targets (PCT/GB2018/051892); methods for lung cancer detection (PCT/US2017/028031); and identifying responders to cancer treatment (PCT/GB2018/051912). C.S. is a Royal Society Napier Research Professor (RP150154). His work is supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001169), the UK Medical Research Council (FC001169) and the Wellcome Trust (FC001169). C.S. is funded by Cancer Research UK (TRACERx, PEACE and CRUK Cancer Immunotherapy Catalyst Network), Cancer Research UK Lung Cancer Centre of Excellence (C11496/A30025), the Rosetrees Trust, the Butterfield and Stoneygate Trusts, NovoNordisk Foundation (ID16584), a Royal Society Professorship Enhancement Award (RP/EA/180007), the National Institute for Health Research (NIHR) Biomedical Research Centre at University College London Hospitals, the Cancer Research UK-Medical Research Council London Centre, the Experimental Cancer Medicine Centre, the Breast Cancer Research Foundation (BCRF) and a Stand Up To Cancer-LUNGevation American Lung Association Lung Cancer Interception Dream Team Translational Research Grant (SU2C- AACR-DT23-17). Stand Up To Cancer is a program of the Entertainment Industry Foundation. Research grants are administered by the American Association for Cancer Research, the Scientific Partner of SU2C. C.S. receives funding from the European Research Council (ERC) under the European Union's Seventh Framework Programme (FP7/2007-2013) Consolidator Grant (FP7-2013-ERC-301971). His work is supported by the Australasian Gastro-Intestinal Trials Group/Merck Clinical Research Fellowship. S.B. has worked in an advisory role for Amgen, AstraZeneca, Clovis Oncology, Epiplogen, Gennab, GlaxoSmithKline, Immunogen, Mersana, Merck Sharp & Dohme, Merck Serono, Oncxerna, Pfizer, Tesaro and Roche; has received institution research funding from AstraZeneca; and has received funding support from NIH/NLM/ICR Biomedical Research Centre for Cancer. L.C. has worked in a consulting or advisory role for Eli Lilly, Bristol Myers Squibb, Merck Sharp & Dohme, Bayer, Roche, Merck Serono, Five Prime Therapeutics, AstraZeneca, Oncxerna, Pierre Fabre, Boehringer Ingelheim, Incyte and Astellas; has received research funding from Eli Lilly and Janssen-Cilag; and has received honoraria from Eli Lilly and Eisai. S.T. has received speaking fees from Roche, AstraZeneca, Novartis and Ipsen. G.K. receives core funding from the Francis Crick Institute (FC0010099). S.T. is funded by Cancer Research UK (grant ref. no. CS0947/1/A18176), the Francis Crick Institute (which receives its core funding from Cancer Research UK (FC0010988), the UK Medical Research Council (FC0010988) and the Wellcome Trust (FC0010988)), the National Institute for Health Research Biomedical Research Centre at the Royal Marsden Hospital and the Institute of Cancer Research (grant ref. no. A109), the Royal Marsden Cancer Charity, the Rosetrees Trust (grant ref. no. A2204), Veufala Medical Systems (grant ref. nos. 10467 and 10530), the National Institutes of Health and the Melanoma Research Alliance. All other authors declare no financial conflicts of interest.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-021-01387-6.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01387-6.

Correspondence and requests for materials

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Extended Data Fig. 1 | Gating strategy for T-cell assay. a, CD4+ T-cell response to SEB in exemplary samples. b, CD8+ T-cell response to SEB in exemplary samples. c, Gating strategy for flow analysis of T-cell activation. PBMCs were stimulated with SEB for 24 hrs. SEB, Staphylococcal enterotoxin B. d, IFN-γ ELISpot at day 17 post vaccine. PBMCs were stimulated with S, S1 and S+ peptide pools representing the full length of the spike protein. Anti-CD3 antibody was used as a positive control (PC), no stimulation was added to the negative control (NC).
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Antibody response to BNT162b2 according to SERA and PIWAS. a, Pre-and post-vaccination antibody levels against SARS-CoV-2 antigenic motifs. b, PIWAS values pre- and post-vaccination against non-spike sequences of the SARS-CoV-proteome compared to patients with SARS-CoV-2 positive subjects24 (Methods). Box plots of PIWAS values of SARS-CoV-2 positive subjects are shown, where boxes denote the IQR, and the median is shown as horizontal bars. Whiskers extend to 1.5x IQR and outliers are shown as individual points (n = 230 subjects; n = 230 biologically independent samples). IQR, interquartile range; PIWAS, protein-based immunome wide association study; SERA, serum epitope repertoire analysis.
Extended Data Fig. 3 | Anti-PF4 antibodies after vaccination. Serum samples collected after admission were analysed for presence of anti-PF4 antibodies by ELISA. Values represent mean of two technical replicates, error bars represent standard deviation. The dotted line indicates the threshold for a sample to be considered positive (OD 450 nm > 0.4).
Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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  - Give P values as exact values whenever suitable.
- [ ] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [ ] Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

| Data collection | N/A |
|-----------------|-----|
| Data analysis   | All statistical analyses were performed in FlowJo v10.7.1 and GraphPad Prism v9.1.0 |

For manuscripts utilizing custom algorithms or software that are not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All requests for raw and analysed data, materials, and CAPTURE study protocol will be reviewed by the CAPTURE Trials Team, Skin and Renal Clinical Trials Unit, The Royal Marsden NHS Foundation Trust (CAPTURE@rmh.nhs.uk) to determine if the request is subject to confidentiality and data protection obligations. Data and materials that can be shared will be released via a material transfer agreement.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size: This is a case report (n=1).

Data exclusions: No data were excluded.

Replication: For correlative measures, all human specimens underwent quality control (QC) assessments. Only those that passed QC were further analyzed.

Randomization: This is a case report (n=1).

Blinding: This is a case report and thus blinding was not performed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | Involved in the study |
| [ ] Antibodies |
| [ ] Eukaryotic cell lines |
| [ ] Palaeontology and archaeology |
| [ ] Animal and other organisms |
| [ ] Human research participants |
| [ ] Clinical data |
| [ ] Dual use research of concern |
| [ ] Involved in the study |
| [ ] ChiP-seq |
| [ ] Flow cytometry |
| [ ] MRI-based neuroimaging |

Antibodies

Antibodies used: A list of antibodies is provided in Table S1.

Validation: Antibodies for AIM assay were chosen on the basis of previous publication of the assay [Griffoni et al, Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell] References with validation of other primary antibodies used are as follows: Neutralising antibodies [NSP8 and anti-rabbit IgG - Wrobel et al. Antibody mediated disruption of the SARS-CoV-2 spike glycoprotein. Nat Comm.; ELISPOT assay antibodies (https://www.mabtech.com/products/human-ifn-gamma-elsa-pro-kit_3420-1hp-10)].

Human research participants

Policy information about studies involving human research participants

Population characteristics: Single human subject. 58 year old male with history of metastatic mismatch repair deficient colorectal cancer on anti-PD1 monotherapy.

Recruitment: CAPTURE study (NCT03226886)

Ethics oversight: CAPTURE was approved as a substudy of TRACERx Renal (NCT03226886). TRACERx Renal was initially approved by the NRES Committee London - Fulham on January 17, 2012. The TRACERx Renal sub-study CAPTURE was submitted as part of Substantial Amendment 9 and approved by the Health Research Authority on April 30, 2020 and the NRES Committee London - Fulham on May 3, 2020. CAPTURE is conducted in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice and applicable regulatory requirements.
Clinical data

Policy information about clinical studies
All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

| Clinical trial registration | CAPTURE study (NCT03268806) |
|----------------------------|-----------------------------|
| Study protocol             | Request for protocols should be directed to CAPTURE trials unit via CAPTURE@rmh.nhs.uk |
| Data collection            | Data was collected at Royal Marsden hospital, by extract from clinical records approved as per protocol. The participant was recruited on the 13/1/21. |
| Outcomes                   | Vaccine efficacy, immunological parameters, and associations with clinical features presented are exploratory endpoints of this study. Primary endpoint of study is description of population characteristics between SARS-CoV-2 positive and negative cancer patients. Secondary endpoints are differences in overall survival, intensive treatment unit admission rate, anti-cancer treatment received, and immune related adverse events. |

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Whole blood was collected in EDTA tubes (VWR) and stored at 4°C until processing. All samples were processed within 24 hours. Time of blood draw, processing, and freezing was recorded for each sample. Prior to processing tubes were brought to room temperature (RT). PBMC and plasma were isolated by density-gradient centrifugation using pre-filled centrifugation tubes (pluriselect). Up to 30 ml of undiluted blood was added on top of the sponge and centrifuged for 30 minutes at 1000 x g at RT. Plasma was carefully removed then centrifuged for 10 minutes at 4000 x g to remove debris, aliquoted and stored at -80°C. The cell layer was then collected and washed twice in PBS by centrifugation for 10 minutes at 300 x g at RT. PBMC were resuspended in Recovery cell culture freezing medium (Fisher Scientific) containing 10% DMSO.

Instrument
All experiments were run on a Bio-Rad Ze5 flow cytometer running Bio-Rad Everest software v2.4

Software
Data were analysed using FlowJo 10.7.1

Cell population abundance
Cells were not sorted in this study

Gating strategy
Lymphocytes were gated in FSC-A/SSC-A plot, followed by gating for singlets by plotting FSC-A vs. FSC-H. Viable CD3+ cells were identified by plotting CD3 vs. CD14, CD19, and viability dye. Next CD4+ and CD8+ cells were gated and finally CD137+OX40+ cells were identified in the CD4+ population and CD137+CD69+ in the CD8+ population.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.