A randomized, observer-blind, phase 1 study of immune responses to three human adenovirus type 26 vector–based vaccines

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eBioMedicine
2026;127: 106271
Published Online xxx
https://doi.org/10.1016/j.ebiom.2026.106271

Summary

Background Adenovirus 26 (Ad26) vector-based vaccines have shown immunogenicity and safety in various infectious disease settings. However, the impact of each vaccine component (vector/insert) on the immune response has yet to be explored in a controlled setting across multiple vaccines.

Methods In this randomised, observer-masked, multicentre phase 1 study, participants aged 18–59 years were assigned to receive one of three vaccine regimens: Ad26.COV2.S (5×10^{10} viral particles [vp]), Ad26.RSV.PreF/Protein (1×10^{11} vp + 150 μg RSV preF protein), or the Ad26.ZEBOV (5×10^{10} vp), MVA-BN-Filo (1×10^{8} InfU) vaccine regimen. The primary endpoint was the change in cytokines, chemokines, and other innate immune response mediators. Transcriptomics and proteomics analyses were also performed.

Findings From October 2022–August 2023, 160 participants received a study vaccine (Ad26.COV2.S, n = 66; Ad26.RSV.PreF/Protein, n = 48; Ad26.ZEBOV, n = 46). Median age was 35·5 years. All vaccines were well tolerated. Across vaccines, cytokine levels peaked on Day 2 and returned to baseline by Day 8. Highest geometric mean increases were observed with Ad26.RSV.PreF/Protein vaccine, followed by Ad26.COV2.S and Ad26.ZEBOV. The Ad26.RSV.PreF/Protein vaccine elicited the most differentially expressed genes and proteins, while Ad26.ZEBOV had the fewest. All vaccines induced strong innate responses associated with innate and antiviral immunity, hallmarked by interferons. Transcriptional responses were consistent among vaccines, differing primarily in magnitude rather than genes expressed.

Interpretation All three vaccine regimens induced specific immunogenic responses, were well tolerated, and triggered strong innate and antiviral immunity responses within 2 days post-vaccination, with no distinct immune signature observed across vaccines per transcriptomics/proteomics analyses.

Funding This study was funded by Johnson & Johnson.

Keywords: Ad26 vector-based vaccines; Immunogenicity; Transcriptomics; Proteomics

Introduction

Vaccine-elicited immune responses can be evaluated using a systems-based approach to characterise the complex interaction between individual components of the immune system.[1,2] Previous studies have profiled responses to a range of vaccines, vaccine platforms, and populations via post hoc analyses of preexisting ribonucleic acid sequencing (RNA-seq) datasets, often with the goal of identifying early markers that correlate with immunogenicity.[3–7] However, the use of preexisting datasets comes with limitations, such as the potential for missing or insufficient samples and lack of control over confounding variables, such as population differences, batch effects and technology used. Additionally, many of these studies examined immune responses to single vaccines, thus limiting the contextualisation of findings.

The adaptive immune response is primed by antigen-presenting cells that detect antigens via pattern

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Research in context

Evidence before this study

We conducted a PubMed search for research articles published from database inception to 17 November, 2025, using key search terms "adenovirus 26" (Ad26) AND "vaccine" with no language restrictions. Search results were then filtered to display "randomised clinical trials" only. A total of 60 articles were retrieved. Of the articles retrieved, eight articles investigated the Ad26-vectored respiratory syncytial virus (RSV) vaccine, four investigated the Ad26-vectored COVID-19 vaccine, and seven investigated the Ad-26-vectored Ebola vaccine. However, no article reported on the simultaneous evaluation of two or more Ad26 vector-based vaccines. Furthermore, when applying the search terms "proteomics OR transcriptomics," no results were retrieved, thus, emphasising the gap in current literature among Ad26 vector-based vaccines, specifically the lack of 'omics data as they pertain to innate immune, cytokine and chemokine responses. After conducting another exploratory search, using search terms "transcriptome" AND "vaccine" and filtering for clinical trials, 34 articles were retrieved, among which three articles reported transcriptomic data on vaccines within comparable disease settings. One article reported on immune responses following SARS-COV-2 vaccination using samples from two prior randomised placebo-controlled trials. In this study, inflammatory signatures were attenuated and B cell-related and CD4 T cell-related pathways were induced, ameliorating the molecular response to subsequent SARS-COV-2 infection. This evidence suggested that labelling breakthrough infections as "vaccine failures" is a misnomer, given the observed molecular impact. One article reported data from a phase 1 study of an inactivated SARS-COV-2 vaccine in Chinese adults and reported a vast diversity of genes related to the vaccine-induced immune response. The remaining article describes a set of phase 1 studies compared immune responses to two Modified Vaccinia Ankara (MVA)-based SARS-CoV-2 vaccine candidates (encoding either the native or a prefusion-stabilised spike) with responses elicited by licenced ChAd and mRNA vaccines, examining longitudinal innate and early adaptive signatures. The prefusion-stabilised insert induced stronger early transcriptional activation and higher neutralising antibody titres, with innate signatures correlating with improved adaptive responses, suggesting that both platform and antigen conformation shape innate signals that influence final immunogenicity. This study confirms that the insert of a vaccine can have an impact on the ensuing immune responses. Of note, when removing all filters, a substantial number of articles were retrieved, including one article in which a comprehensive transcriptional analysis and comparison of 13 different vaccines and their antibody responses was reported. However, this study was done post hoc with samples from previously published studies. Therefore, overall, as per our original and exploratory searches, very few studies have yet compared the 'omics response to multiple vaccines with comparable vectors, simultaneously, in a real-time, controlled, clinical setting.

Added value of this study

In this randomised, observer-masked, multicentre phase 1 study, we evaluated (within a controlled, clinical setting) the innate, proinflammatory and other pathway responses of three Ad26 vector-based vaccines with varying vaccine inserts, using comprehensive transcriptomic and proteomic analyses, to explore how immune responses may be influenced by the vaccine insert. To our knowledge, no study has directly compared three vaccines with the same backbone. We present detailed transcriptional and proteomic data to provide a more in-depth, mechanistic insight into the role of specific proteins and genes in the elicited immune response.

Implications of all the available evidence

Data from transcriptomic and proteomic analyses demonstrated that the immune responses elicited by the different Ad26-based vaccines were highly similar, while the magnitude of expression of individual genes and proteins differed somewhat between vaccines. All three vaccines induced strong innate responses that were associated with innate and antiviral immunity, hallmarked by type I and II interferons and other cytokines and chemokines. Furthermore, we demonstrated that innate immune responses primarily peaked at Day 2 across all Ad26 vaccines, indicative of an early, robust response to vaccination. The data described in this report suggest that the responses to Ad26-vectored vaccines are not obviously influenced by the insert, with no distinct immune signature, and may follow general patterns of viral vector-based vaccines. Collectively, these data provide valuable insight into the intricate molecular mechanisms of Ad26 vector-based vaccines.

recognition receptors (PRRs), subsequently instructing T and B cells and other immune cells to respond accordingly.[8,9] The antigen itself may also influence the instructions received by T cells based on the cytokines produced by downstream PRR signalling. To date, this multifaceted interplay has not been measured in vaccine trials in a controlled, clinical setting, as comparisons are commonly performed using existing data from different clinical trials that vary in study design and population.[3]

Here, we present data from a unique clinical study (RSV2008) using comprehensive proteomic and transcriptomic profiling to compare early immune responses to a single dose of one of three Ad26 vector-

based vaccines with inserts for SARS-COV-2, respiratory syncytial virus (RSV), or Zaire ebolavirus (Ad26.COV2.S, Ad26.RSV.PreF/Protein, and Ad26.ZEBOV, respectively) at several time points after vaccination, and to explore how the type of response (ie, type of proteins, genes, or transcription modules regulated) may be influenced by the vaccine insert. Furthermore, we validate and expand upon our RNA-seq findings using a dataset from a separate study (COV2008; ClinicalTrials.gov Identifier: NCT04999111), in which an Ad26.COV2.S vaccine was administered either as a homologous or heterologous booster dose following primary vaccination with Ad26.COV2.S or BNT162b2, a messenger ribonucleic acid (mRNA) COVID-19 vaccine.[10]

Methods

Study design and ethics

The randomised, observer-masked, multicentre phase 1 RSV2008 study was conducted at seven sites in the United States and Brazil between October 2022 and August 2023. The study protocol and all amendments were approved by an independent ethics committee or institutional review board at each participating study site. The trial was registered with REBEC (UTN code: U1111-1279-5964). The study was conducted in accordance with the International Council of Harmonisation guidelines on Good Clinical Practice and the Declaration of Helsinki, and all study participants provided written informed consent. The clinical trial protocol and statistical analysis plan are included in the Supplemental Information. An internal Data Review Committee (DRC) was established to address any safety issues that arose during the study. Additionally, and institutional review board approved of the study (Sterling IRB ID: 10331-*MASTER, Advarra USA: IBC NUMBER: PROTO202200131).

At the time of the study, Ad26.COV2.S was authorised for human use, having received Emergency Use Authorisation from the U.S. Food and Drug Administration (FDA) and conditional marketing authorisation from the European Medicines Agency (EMA). The Ad26.RSV.preF–RSV preF protein vaccine was an investigational product approved for testing in humans as an investigational new drug (IND). The safety and efficacy of this vaccine has been evaluated in multiple clinical trials, including VAC18193RSV2001 (NCT03982199).[11,12] The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is authorised for human use by the EMA.

Participants

Participants aged 18–59 years with a body mass index of <35 kg/m^2 were eligible for study participation; those with stable and well-controlled medical conditions were permitted to participate. Individuals with a history of malignancy within the previous 5 years, abnormal immune system function, or allergy/anaphylaxis to vaccines were excluded. There were no exclusions based on race or ethnicity; see Supplemental Methods for full inclusion and exclusion criteria.

Randomisation and masking

Participants were randomised in a 2:1:1 ratio to one of three vaccine groups: Ad26.COV2.S, Ad26.RSV.PreF/Protein, or Ad26.ZEBOV. Randomisation was stratified by baseline SARS-COV-2 serostatus with different randomisation ratios within SARS-COV-2 serostatus strata to increase the likelihood of including a sufficient number of seronegative participants in the Ad26.COV2.S group, while maintaining the 2:1:1 ratio overall. However, by the time of study enrolment, due to the availability of COVID-19 vaccines and emergence of the highly transmissible SARS-COV-2 Omicron (B.1.1.529) variant, the feasibility of enrolling SARS-COV-2 seronegative individuals was greatly impacted. Therefore, per protocol amendment 2 (13 January 2023), minimum/maximum limits for SARS-COV-2 seropositive participant enrolment and requirement for stratification by baseline serostatus were removed, which led to the following ratio of recruitment into the three groups: (Ad26.COV2.S, n = 66; Ad26.RSV.PreF/Protein, n = 48; Ad26.ZEBOV, n = 46). Randomisation was performed centrally using a computer-generated schedule prepared prior to study initiation by or under the supervision of the study sponsor, balanced using randomly permuted blocks. Vaccine recipients and those responsible for the evaluation of study endpoints were masked to vaccine assignment until Day 29, whereas those responsible for study vaccine administration were unmasked. Following unmasking, participants were followed up through 6 months of open label follow-up. Participants who received Ad26.RSVPreF/protein vaccine or Ad26.ZEBOV on Day 1 were offered a single dose of Ad26.COV2.S on Day 29 (optional) as the study was ongoing during the COVID-19 pandemic.

Procedures

A single dose of vaccine was administered intramuscularly (into the deltoid) on Day 1 to participants in the Ad26.COV2.S (5×10^{10} viral particles [vp]) and Ad26.RSV.PreF/Protein (1×10^{11} vp with 150 μg prefusion conformation of RSV F [RSV preF] protein) groups; participants in the Ad26.ZEBOV group received Ad26.ZEBOV (5×10^{10} vp) on Day 1 and MVA-BN-Filo (1×10^{8} Inf U) on Day 57. The molecular design and antigen expression constructs of the vaccines have been described before, Ad26.COV2.S in Bos et al., Ad26.RSV.PreF/Protein in Williams et al., and the Ad26.ZEBOV and MVA-Filo in Milligan et al.[13–15]

Endpoints

The primary endpoint was the change from baseline in cytokines, chemokines, and other protein mediators of

the innate immune response to vaccination with Ad26-vectored vaccines. Key secondary endpoints included safety and reactogenicity and humoral immune responses. Exploratory endpoints included the evaluation of mRNA expression levels related to vaccine-induced innate and proinflammatory responses. Proteins involved in the immune response were also evaluated.

Immune response assessments

Immunogenicity evaluations included enzyme-linked immunosorbent assays (ELISAs), as described in previous studies, of SARS-COV-2 S-specific protein-binding antibodies,[16] RSV-A preF protein-binding antibodies,[17] and Ebola virus (EBOV) glycoprotein-binding antibodies.[18] Insert-specific ELISAs were conducted separately for each vaccine group, and were not normalised to each other. An adenovirus neutralisation assay was used to evaluate neutralising antibody (nAb) responses against the Ad26 vector. Blood samples to evaluate humoural immune responses were collected at baseline (Day 1, prevaccination), Day 8, Day 29, and Day 183 (approximately 6 months post vaccination [post first vaccination for Ebola vaccine, 126 days post the MVA-Filo dose at Day 57]). Innate response mediators using blood plasma samples were measured at baseline and Days 2, 4, 8, and 29. Chemokines, cytokines, and other proteins in plasma were measured by multiplexed assays, specifically Olink® Target 48, a multiplexed proteomics platform based on quantitative polymerase chain reaction.[19]

Safety assessments

Adverse events (AEs) were reported for the duration of the study. Participants recorded solicited local and systemic signs and symptoms for 7 days after first vaccination; unsolicited AEs were recorded through Day 29. Serious AEs (SAEs), AEs of special interest (AESIs), and AEs leading to study discontinuation were reported throughout until completion of a participant's last study-related procedure. The AESI in this study was thrombosis with thrombocytopaenia syndrome (TTS), which is a very rare disorder that has been reported following immunisation with Ad26.COV2.S.[20] Suspected AESIs were recorded from the moment of vaccination until 6 months post-vaccination or early withdrawal. An AESI Adjudication Committee with appropriate expertise was established to evaluate each suspected AESI and determine whether it was a case of TTS (Supplemental Methods).

Transcriptomics and proteomics

RNA extraction and next-generation sequencing (NGS) was performed by Eurofins. Total RNA was extracted from 4 mL whole blood samples collected on Days 1, 2, 4, 8, and 29 in PAXgene® tubes. RNA quantification (Invitrogen Qubit) and quality control (QC) assessments (Agilent Bioanalyzer) were performed. Isolated RNA that passed QC with sufficient quantity/quality was brought forward for library preparation. Libraries were quantified, QC checked, normalised, and paired-end sequenced (Illumina NovaSeq), with 150 base pair reads and a target of 40 million read pairs (80 million total reads) per sample. For RNA-seq read mapping, merged FASTQ reads were trimmed and mapped to the Ensembl GRCh38 primary assembly of the human genome. Quantification was performed using RNA-seq by expectation maximisation, and expected counts per gene were used for downstream analysis. An expression set (Eset) was then generated and consisted of information on genes, samples, grouping, and expression matrix. The Eset served as the primary input used to analyse transcriptional variability and differentially expressed genes (DEGs). Briefly, genes with low expression were filtered out before quantifying the expression level of each transcript. Principal component analysis (PCA) was performed to evaluate clustering of data. Fold changes in gene expression were estimated between time points and between groups. A gene was considered to be differentially expressed if the false discovery rate (FDR)-corrected p-value was <0·05, with an absolute fold change of >1·5 (ie, absolute $\log_2$ fold change >0·58, FDR <0·05) from baseline (up- or downregulated). To annotate DEGs, a blood transcription module (BTM) analysis was performed, generating fingerprint plots to allow for the comparison of all responses across the transcriptome.[21] A gene set variation analysis (GSVA) was also performed to support the BTM analysis. GSVA is a gene set enrichment analysis, functioning on the single sample level and using gene sets, rather than individual genes, as the functional unit to estimate gene set activity.

For proteomics, the Olink® Explore 3072 assay was used to generate proteomic profiles of each plasma sample. This assay included ~3000 proteins and used NGS to detect and quantify protein concentration in arbitrary units of normalised protein expression, which was used to calculate fold changes from baseline or between samples. Downstream analyses for proteomics were similar to those described above and included analyses of variability and differentially expressed proteins (DEPs). A protein was considered to be differentially expressed if the FDR-corrected p-value was <0·05, with an absolute fold change of >1·5 (ie, absolute $\log_2$ fold change of >0·58) from baseline (up- or downregulated) across vaccine groups and time points (nine comparisons in total). Additional methodology is provided in the Supplemental Methods.

COV2008 RNA-seq study

To investigate the role of early immune responses to Ad26 vector-based vaccines, a dataset from a separate randomised, double-blind, phase 2 study (COV2008; ClinicalTrials.gov Identifier: NCT04999111) was

analysed.[10] In this study, Ad26.COV2.S was administered as a booster vaccination at different dose levels in participants who had previously received primary vaccination with either a single dose of Ad26.COV2.S (Cohort 1) or two doses of BNT162b2 (Cohort 2).[10] Therefore, both cohorts differed in number and type of previous exposures, resulting in differences in baseline immunity against the SARS-COV-2 spike protein and the Ad26 vector. For a subset of study participants (~30 per group), transcriptomic responses were sequenced at baseline (ie, before booster vaccination with Ad26.COV2.S) and at Day 2 or Day 8 after booster vaccination with either $1{\cdot}25 \times 10^{10}$ vp (low dose) or 5×10^{10} vp Ad26.COV2.S (standard dose).

Analysis sets and statistical analysis

The full analysis set included all participants with ≥1 documented vaccine administration; all safety and participant demographic/characteristic analyses were conducted on the full analysis set. To evaluate insert-specific immunogenicity, group-specific per-protocol immunogenicity sets were defined for each group, which included participants who received the respective study vaccine and had immunogenicity data available. Samples following a major protocol deviation expected to impact immunogenicity were excluded. For non-vaccine-specific evaluations of immune responses, a corresponding per-protocol immunogenicity set was defined.

Missing data were not imputed. Analyses were generally based on observed data only. Values below the lower limit of quantification (LLOQ) or above the upper limit of quantification (ULOQ) were handled according to prespecified rules, including imputation to LLOQ/2 or ULOQ, as appropriate, for calculation of geometric means and fold changes.

To sufficiently explore innate immune response and immunogenicity, with a randomisation ratio of 2:1:1, 80 participants were to be enrolled and allocated to the Ad26.COV2.S vaccine, 40 to the Ad26.RSV.PreF/Protein vaccine, and 40 to the Ad26.ZEBOV vaccine, for a total sample size of 160 participants. Of the 80 participants allocated to the Ad26.COV2.S vaccine group, 40 were to be SARS-COV-2 seropositive and 40 were to be SARS-COV-2 negative at baseline. However, per-protocol amendment 2 (as described above), the SARS-COV-2 seropositive requirement was lifted, and flexibility was allowed to help meet enrolment targets. Given the exploratory nature of this study, sample sizes were selected to provide an initial understanding of innate immune and immunogenicity responses. No formal power calculation was performed. The chosen sample size was intended to support descriptive analyses of gene expression profiles across groups and was informed by preliminary data indicating that a minimum of 20 participants per group could achieve approximately 80% statistical power.

No formal statistical hypothesis testing was conducted. Continuous variables, including primary endpoints such as cytokines, chemokines, and other protein mediators of the innate immune response, in addition to proinflammatory and other pathway responses to Ad26 vaccination, were summarised descriptively. Geometric means and geometric mean increases (GMI) from baseline, each with 95% confidence intervals were reported. Frequencies of binary endpoints including vaccine-specific definitions of responders and participants achieving 2- and/or 4-fold increases in binding antibodies, were also presented. In the analyses of the transcriptional and proteomic responses, the FDR-adjusted p-values were used as a cut off for classifying genes and proteins as 'differentially expressed'.

All analyses were prespecified in the statistical analysis plan.

Role of funding source

This work was funded by Johnson & Johnson during all stages of the trial, including its design, data collection, analysis, and the development and publishing of the manuscript, including scientific writing assistance and statistical analyses.

Results

Participants

In total, 215 participants were screened, and 160 were randomised and vaccinated (Ad26.COV2.S, n = 66; Ad26.RSV.PreF/Protein, n = 48; Ad26.ZEBOV, n = 46; Fig. 1). Median participant age was 35·5 years (interquartile range, 29·0–45·5) and baseline characteristics were generally well balanced over the three vaccine groups (Supplemental Table S1). No sex- or gender-based analyses were performed.

Safety

Safety outcomes are summarised in Supplemental Table S2, with solicited and unsolicited AEs reported in Table 1. A higher proportion of participants in the Ad26.RSV.PreF/Protein group reported AEs compared with the Ad26.COV2.S and Ad26.ZEBOV groups. Grade 3 solicited AEs were higher in the Ad26.RSV.-PreF/Protein group compared with the Ad26.COV2.S and Ad26.ZEBOV groups, driven primarily by a higher frequency of grade 3 solicited systemic AEs (10/46 [21·7%] participants, compared with 3/64 [4·7%] and 1/46 [2·2%] participants, respectively). All solicited local AEs and systemic AEs were transient and resolved with a median duration of 2–5 days and 1–2 days, respectively. Serious AEs occurred in 4/160 (2·5%) participants; none were considered vaccine-related. Potential AESIs were reported by 3/160 (1·9%) participants, none of which were serious or qualified for assessment by the TTS adjudication committee.

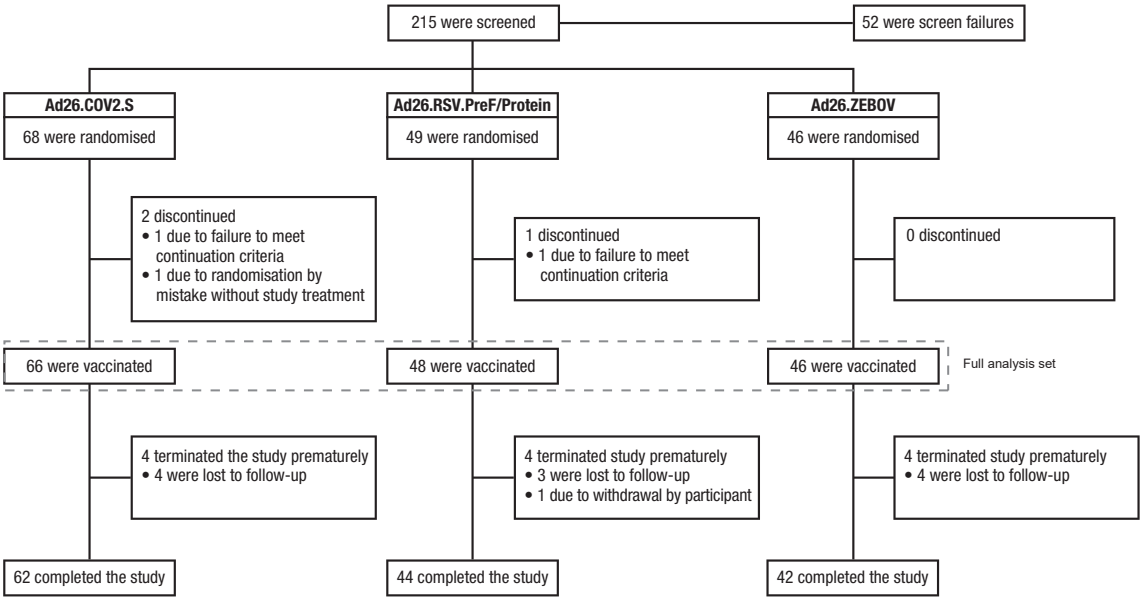

Fig. 1: **Participant disposition.** A total of 160 participants were included in the full analysis set, defined as participants with one or more vaccine administration documented, regardless of protocol deviations or vaccine type (Ad26.COV2.S, n = 66; Ad26.RSV.PreF/Protein, n = 48; Ad26.ZEBOV, n = 46). While not shown, a total of 158 participants were included in the per-protocol immunogenicity set, defined as all randomised participants who received a study vaccine on Day 1 and for whom immunogenicity data were available (Ad26.COV2.S, n = 65; Ad26.RSV.PreF/Protein, n = 47; Ad26.ZEBOV, n = 46). 12 participants in the Ad26.RSV.PreF/Protein group opted to receive the optional Ad26.COV2.S vaccination, and 9 in the Ad26.ZEBOV group. Ad26, adenovirus type 26; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus.

Immunogenicity

Insert-specific ELISAs were conducted separately for each vaccine group and were not normalised against each other. Binding antibodies against SARS-COV-2 and RSV-A preF protein were present at baseline in the Ad26.COV2.S (geometric mean concentration, 9886 ELISA units/mL) and Ad26.RSV.PreF/Protein (267 ELISA units/mL) groups, respectively (Supplemental Table S4–5). Antibodies against EBOV glycoprotein were below the limit of quantification in the

	Ad26.COV2.S (n = 66)	Ad26.RSV.PreF/ protein (n = 48)	Ad26.ZEBOV (n = 46)	Total (N = 160)
Participants, n[a]	64	46	46	156
Participants with ≥1 solicited local AE (7 days postdose), n (%)	40 (62·5)	36 (78·3)	26 (56·5)	102 (65·4)
Pain/tenderness	39 (60·9)	36 (78·3)	26 (56·5)	101 (64·7)
Erythema	3 (4·7)	2 (4·3)	2 (4·3)	7 (4·5)
Swelling	2 (3·1)	2 (4·3)	0	4 (2·6)
Participants with ≥1 solicited systemic AE (7 days postdose), n (%)	40 (62·5)	37 (80·4)	28 (60·9)	105 (67·3)
Fatigue	29 (45·3)	28 (60·9)	16 (34·8)	73 (46·8)
Headache	27 (42·2)	26 (56·5)	16 (34·8)	69 (44·2)
Myalgia	23 (35·9)	26 (56·5)	13 (28·3)	62 (39·7)
Nausea	14 (21·9)	14 (30·4)	3 (6·5)	31 (19·9)
Pyrexia	5 (7·8)	13 (28·3)	0	18 (11·5)
Participants, n	66	48	48	160
Participants with ≥1 unsolicited AE (28 days postdose),[b] n (%)	23 (34·8)	27 (56·3)	9 (19·6)	59 (36·9)
Chills	1 (1·5)	7 (14·6)	2 (4·3)	10 (6·3)
Upper respiratory tract infection	2 (3·0)	4 (8·3)	2 (4·3)	8 (5·0)
Vomiting	0	3 (6·3)	0	3 (1·9)

Ad26, adenovirus type 26; AE, adverse event; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus. Note that participants were counted only once within a period for any given event, regardless of the number of times they experienced the event in that period. [a]The number of participants with diaries assessed by the principal investigator. [b]AEs reported in at least 5% of the participants in any group.

Table 1: **Solicited and unsolicited adverse events after vaccination (full analysis set).**

Ad26.ZEBOV group at baseline (Supplemental Table S6). Geometric mean concentrations of binding antibodies increased from baseline to Day 29 in all groups, with the highest GMI observed in the Ad26.ZEBOV group, likely due to the lower baseline values, followed by Ad26.RSV.PreF/Protein, and Ad26.COV2.S. GMIs remained above baseline at Day 183, with a small increase observed from Day 29 to Day 183 in the Ad26.ZEBOV group, due to the receipt of MVA-BN-Filo vaccine on Day 57.

At baseline, in the Ad26.COV2.S, Ad26.RSV.PreF/Protein, and Ad26.ZEBOV groups, Ad26 nAb titres above the limit of detection were present in 26·2%, 25·5%, and 13·0% of participants, respectively (Supplemental Table S7). On Day 29, the GMI (in titres) for these three groups were 17·7, 15·5, and 11·3, respectively, with all GMIs decreasing by Day 183. Preexisting nAbs against the Ad26 vector had no apparent effect on immunogenicity, with no correlation observed between baseline Ad26 nAb titres and insert-specific binding antibody levels following Ad26.COV2.S or Ad26.RSV.PreF/Protein vaccination. Too few participants in the Ad26.ZEBOV group had detectable Ad26 titres at baseline to draw any meaningful conclusion.

Cytokine profiling

Changes from baseline in observed cytokines across vaccine groups are shown in Fig. 2. The largest GMI from baseline for all vaccine groups was observed at Day 2, followed by a decrease by Day 4, and returning to (or near to) baseline levels by Day 8. Most cytokines that were upregulated on Day 2 for all vaccine groups were inflammatory cytokines, including interferon-gamma (IFN-γ), IL-6, CXCL10, CXCL11, CCL2, CCL3, CCL4, CCL7, CCL8, IL-15, and TNF-α (all with an observed GMI of ≥1·5). Of these, the highest upregulated (greatest GMI) cytokines at Day 2 across all vaccine groups were IFN-γ (Ad26.RSV.PreF/Protein, GMI 138·6; Ad26.COV2.S, 53·0; Ad26.ZEBOV, 18·3), followed by CXCL10 (GMI 32·4; 13·8; 8·7). In addition, CXCL9, which assists in immune cell migration, and CCL19, a leucocyte chemoattractant, were also upregulated from baseline at Day 2 for all vaccine groups. A similar observation was made for the anti-inflammatory cytokine IL-10, as well as IL-27, which promotes anti-inflammatory effects and regulates Th1/Th2 cell differentiation. Although similar responses were observed across the three vaccine groups, responses were generally the highest in the Ad26.RSV.PreF/Protein group and lowest in the Ad26.ZEBOV group.

Proteomic responses

The total number of up- or downregulated significant DEPs compared with baseline by vaccine group, identified using Olink® analysis, is shown in Fig. 3a. The highest number of all DEPs from baseline were observed with Ad26.RSV.PreF/Protein, peaking at Day 2, decreasing by Day 4, further declining by Day 8, and returning close to baseline by Day 29. In contrast, responses in the Ad26.COV2.S group were lower, peaking at Day 4 from baseline. The lowest DEP response was observed in the Ad26.ZEBOV group.

Per PCA of proteomic responses, the strongest vaccine effects occurred at Day 2 and Day 4 (Supplemental Figure S1). No obvious clustering of the vaccine groups was observed. When responses from each vaccine group were pooled and compared with baseline levels, the strongest and most significant fold changes in DEPs from baseline (up or down) for each group occurred at Days 2 and 4, decreased at Day 8, and had returned to baseline at Day 29 (Supplemental Figure S2). Although slightly lower fold changes were observed in the Ad26.ZEBOV group, response kinetics were similar across all vaccine groups.

DEPs that had at least a 2-fold increase or decrease at any time point from baseline ($\log_2$ fold change of ≥1) are presented in Fig. 3b. Significant changes from baseline were primarily observed at Days 2, 4, and 8, with strongest responses occurring at Day 2 for all vaccine groups. Similar to the cytokine analysis, inflammatory cytokines were observed to be upregulated at Day 2 across all three vaccine groups, including IFN-γ, CXCL10, CXCL11, and CCL8.

Although the highest fold-changes for protein upregulation were mainly observed at Day 2 in all vaccine groups, some proteins, including IDO1, GBP1, IFIT3, UBE2L6, CLEC6A, and TYMP, reached peak upregulation on Day 4. At Day 8, only a few proteins were upregulated in all vaccine groups, including TNFRSF8 and DUT. Compared with baseline, 10 proteins were consistently downregulated in all three vaccine groups at Days 2, 4 and 8 (Supplemental Figure S3).

Transcriptional responses

Transcriptional responses were similar across vaccine groups, with the strongest responses from baseline observed at Day 2, decreasing by Day 4, further declining by Day 8, and returning to (or near to) baseline levels by Day 29 (Fig. 4a). Per PCA of transcriptomic responses, the strongest responses occurred on Day 2 in all vaccine groups (Supplemental Figure S4). Compared with baseline, most genes were upregulated, fewer genes were downregulated, and greater fold changes in either direction were observed at Day 2 across all vaccine groups (Supplemental Figure S5). The top 50 significant DEGs for the Ad26.COV2.S, Ad26.RSV.PreF/Protein, and Ad26.ZEBOV groups are summarised in Supplemental Tables S8–10, respectively. Three of the top DEGs expressed at each time point across vaccine groups are depicted in Fig. 4b. At Day 2, most DEGs were related to the innate immune response, inflammation, and

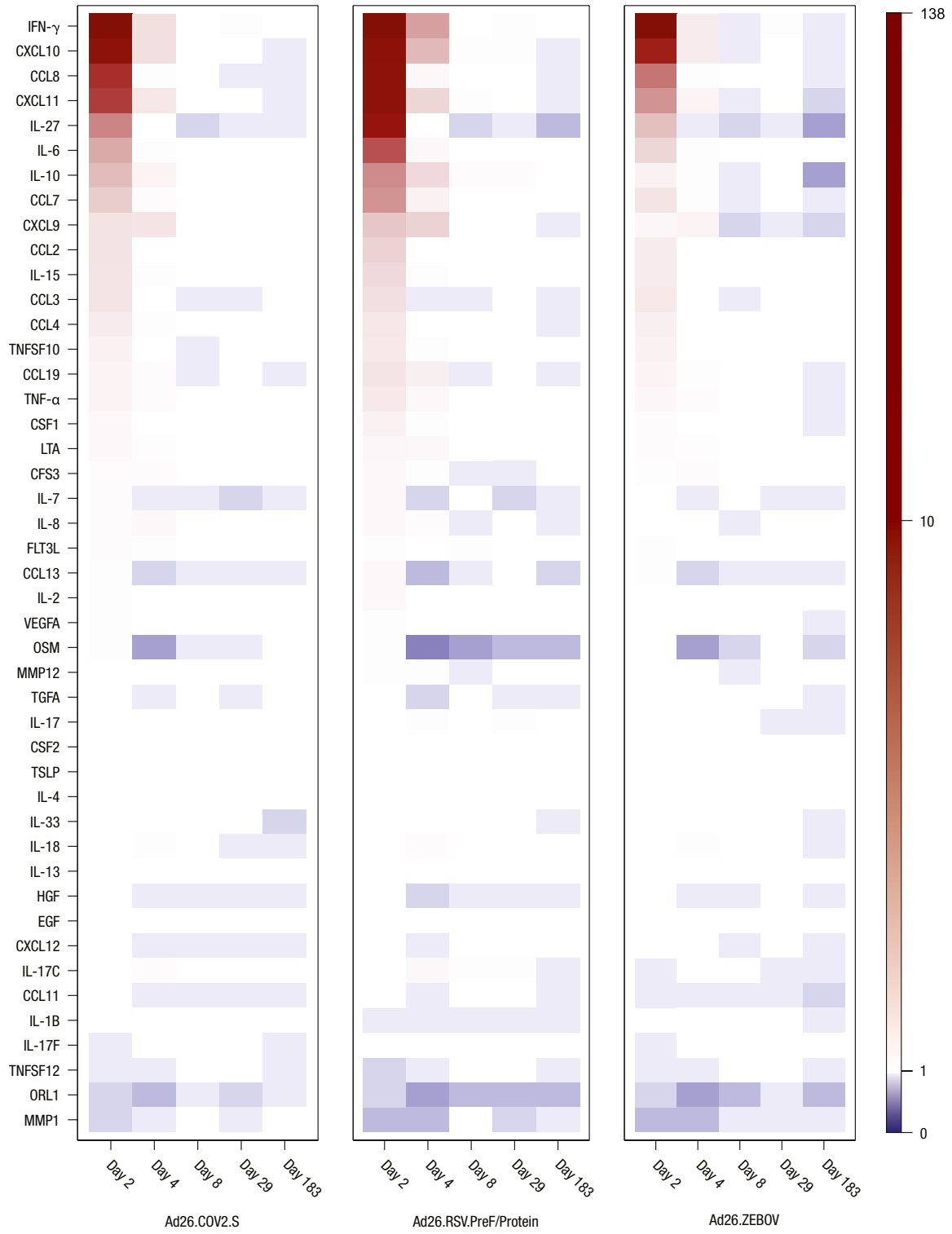

Fig. 2: **Cytokine profiling by Ad26 vaccine and time point.** The heatmap depicts geometric mean increase or decrease from baseline (prevaccination) in cytokines identified using an Olink® proteomics analysis. A darker, saturated red colour indicates a greater geometric mean increase; a darker, saturated blue colour indicates a greater geometric mean decrease. Ad26, adenovirus type 26; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus.

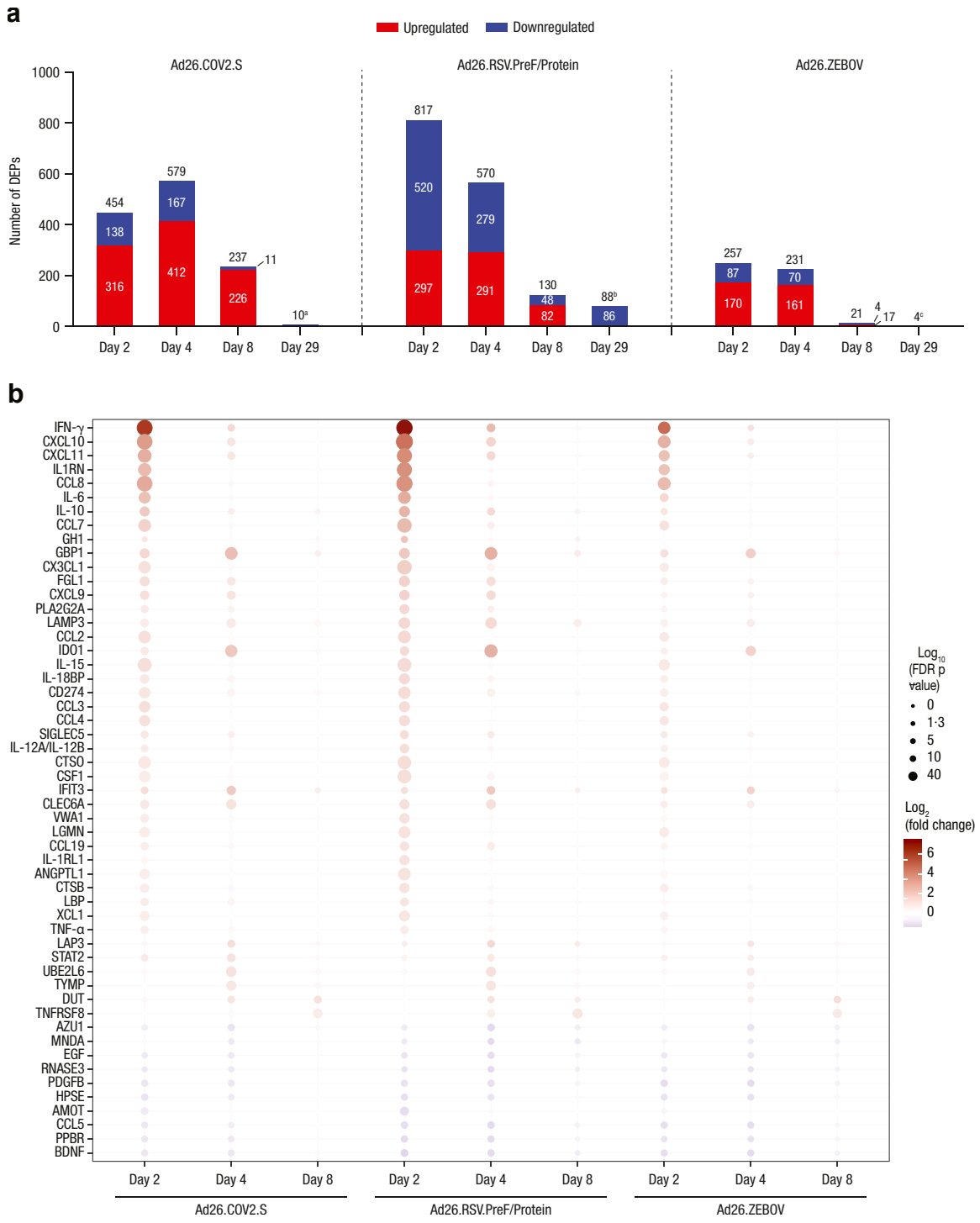

***Fig. 3*: Overview of differentially expressed proteins by Ad26 vaccine group and time point.** a) shows number of DEPs (with a >1·5 fold change [absolute $\log_2$ fold change of >0·58]) identified using an Olink® proteomics analysis that had an FDR-corrected p-value of <0·05, split by up- and downregulation (red and blue, respectively) across all vaccine groups and time points compared with baseline (prevaccination). b) shows top DEPs with an FDR-corrected p-value of <0·05 and ≥2-fold change (absolute $\log_2$ fold change of >1) from baseline (prevaccination). Due to the large number of DEPs identified with a >1·5 fold change (as shown in panel a) a fold change of ≥2 was selected for panel b to allow visualisation of key up- or downregulated DEPs. Increased saturation of colour indicates upregulation, lower saturation indicates downregulation. Ad26, adenovirus type 26; DEP, differentially expressed protein; FDR, false discovery rate; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus. [a]Ten total DEPs, eight upregulated and two down-regulated. [b]88 total DEPs, two upregulated and 86 downregulated. [c]Four total DEPs, all upregulated.

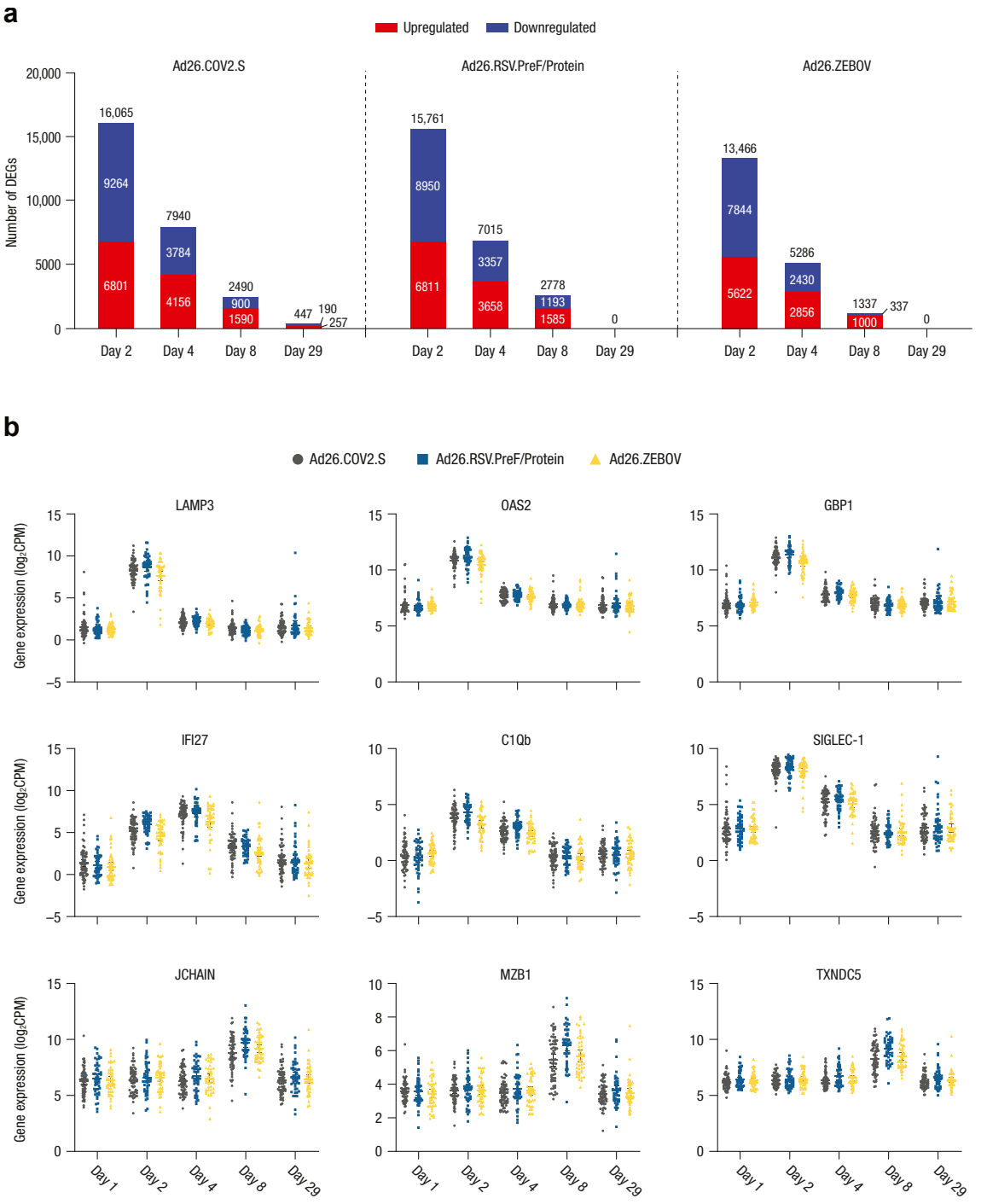

Fig. 4: **Overview of differentially expressed genes by Ad26 vaccine group and time point.** a) shows the number of DEGs identified (with a >1·5 fold change [absolute $\log_2$ fold change of >0·58]) using a transcriptomic analysis that had an FDR-corrected p-value of <0·05, split by up- and downregulation (red and blue, respectively) across all vaccine groups and time points compared with baseline (prevaccination). b) shows plots of three of the top DEGs expressed at each time point across vaccine groups. LAMP3 is involved in phagocytosis, an important component of innate immune cells. OAS2, GBP1, IFI27 and SIGLEC-1 are interferon-specific genes. C1Qb is a component of complement. JCHAIN, TXNDC5 and MZB1 are all B cell related genes. Ad26, adenovirus type 26; DEG, differentially expressed gene; FDR, false discovery rate; CPM, counts per million; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus.

antiviral responses, including interferon-stimulated genes (ISGs). By Day 4, responses still included some ISGs; however, genes related to general inflammation and complement activation were also observed. By Day 8, most upregulated genes were related to B cell responses, antibody production, and cell proliferation.

Given that significant DEGs were defined specifically as those genes either up- or downregulated by a fold change of ≥1·5 (log_2 fold change of 0·58) at any time point from baseline, gene annotation via a BTM analysis was performed only at Day 2 for each vaccine group (Fig. 5). At Day 2, observed responses were strongest in the Ad26.RSV.PreF/Protein group, followed by the Ad26.COV2.S and Ad26.ZEBOV groups. However, up- and downregulated modules were generally similar between groups. The most dominant module response observed across all vaccine groups was related to type I and type II interferons, with modules containing predominantly ISGs, which were also among the top 25 observed DEGs for Day 2 (Supplemental Tables S11–13). In addition to interferons, modules related to monocytes and neutrophils were also upregulated across all vaccine groups; however, responses were less pronounced in the Ad26.ZEBOV group. In contrast, compared with baseline, lymphocyte-related modules were downregulated at Day 2 across all vaccine groups.

Following a GSVA of a selection of the top 25 most significant BTMs in each vaccine group and time point (fold change ≥1·5, FDR-corrected p value of <0·05; Fig. 6), module-responses were observed to be similar across the three vaccine groups. Compared with baseline, at Day 2, several modules related to protein synthesis, cytokines/chemokines, and monocytes were upregulated, whereas B cell, lymphocytes and T cell modules were significantly downregulated. Observed responses at Day 4 were more diverse; while the type I interferon module was still among the top differentially expressed modules, complement and monocyte modules were also upregulated. Several modules related to plasma cells were also upregulated, indicating a detectable humoural response in the transcriptome. These modules were also highly upregulated at Day 8. Compared to other vaccine groups, responses were lower at all time points for the Ad26.ZEBOV vaccine, except at Day 8, at which point plasma modules were equal to, or slightly higher than the other two vaccine groups (Supplemental Figure S6).

COV2008 RNA-seq findings

The innate response to the three vaccine regimens was generally similar in nature but differed in magnitude. These differences could be due to the dose or formulation of the vaccines, or due to the different immunological backgrounds in these groups, with the Ad26.ZEBOV group not having any pre-exposure to the Ebola virus whereas baseline seropositivity was observed in all participants in the Ad26.COV2.S and the Ad26.RSV.PreF/Protein group. To further investigate responses to Ad26-vectored vaccines in differing immunological backgrounds, a subset of samples was analysed from the phase 2 COV2008 study, in which participants received Ad26.COV2.S as a homologous or heterologous booster.[10] An overview of vaccine group assignment for participants included in the subset analysis is provided in Supplemental Table S14. Compared with baseline, the strongest transcriptomic response was observed at Day 2 (1 day after booster vaccination) in both Cohort 1 (primary vaccination with Ad26.COV2.S) and Cohort 2 (primary vaccination with BNT162b2), for both Ad26.COV2.S booster dose levels (Fig. 7a; Supplemental Figure S7). The number of DEGs after the low-dose booster ($1·25 \times 10^{10}$ vp) of Ad26.COV2.S was comparable between the two cohorts. Among the participants who received the standard dose booster (5×10^{10} vp), the number of DEGs was higher in Cohort 2 versus Cohort 1. Compared with baseline, fewer DEGs were observed in Cohort 2 at Day 8 and no DEGs were observed at Day 8 in Cohort 1 with either booster dose level (Supplemental Figure S8).

At Day 2, the most significantly upregulated genes were downstream of type I interferons or IFN-γ and were related to the overall innate immune responses (Fig. 7b). Gene upregulation from baseline was generally higher in Cohort 2, and all responses were booster dose-dependent, with lower levels of differential expression observed with the low versus standard booster dose. When directly comparing responses in Cohort 2 versus Cohort 1 at Day 2 within the standard dose groups, many genes exhibited significantly different magnitudes of expression, such as GRIN3A, while some transcriptional responses were observed in only one of the cohorts (Fig. 7b). Following a BTM pathway analysis of transcriptional responses (Fig. 8), more BTMs were up- or downregulated at Day 2 compared with baseline, hallmarked by interferon-related genes (Fig. 9). At Day 2 among participants in Cohort 2, monocytes and neutrophil activation modules were also upregulated, whereas protein modification, T cells, and prostanoid modules were downregulated. Overall, transcriptional activity at Day 2 was higher in Cohort 2 than in Cohort 1 (Supplemental Figure S9).

Among the 44 most significant modules expressed at Day 2 in Cohort 2 following a standard booster dose, type I interferon responses were the most significantly upregulated, with the highest observed fold changes. Other modules that were increased at Day 2 included those related to cytokines/chemokines, protein synthesis, complement activation, monocytes and neutrophils. At Day 8, responses were dominated by plasma cell

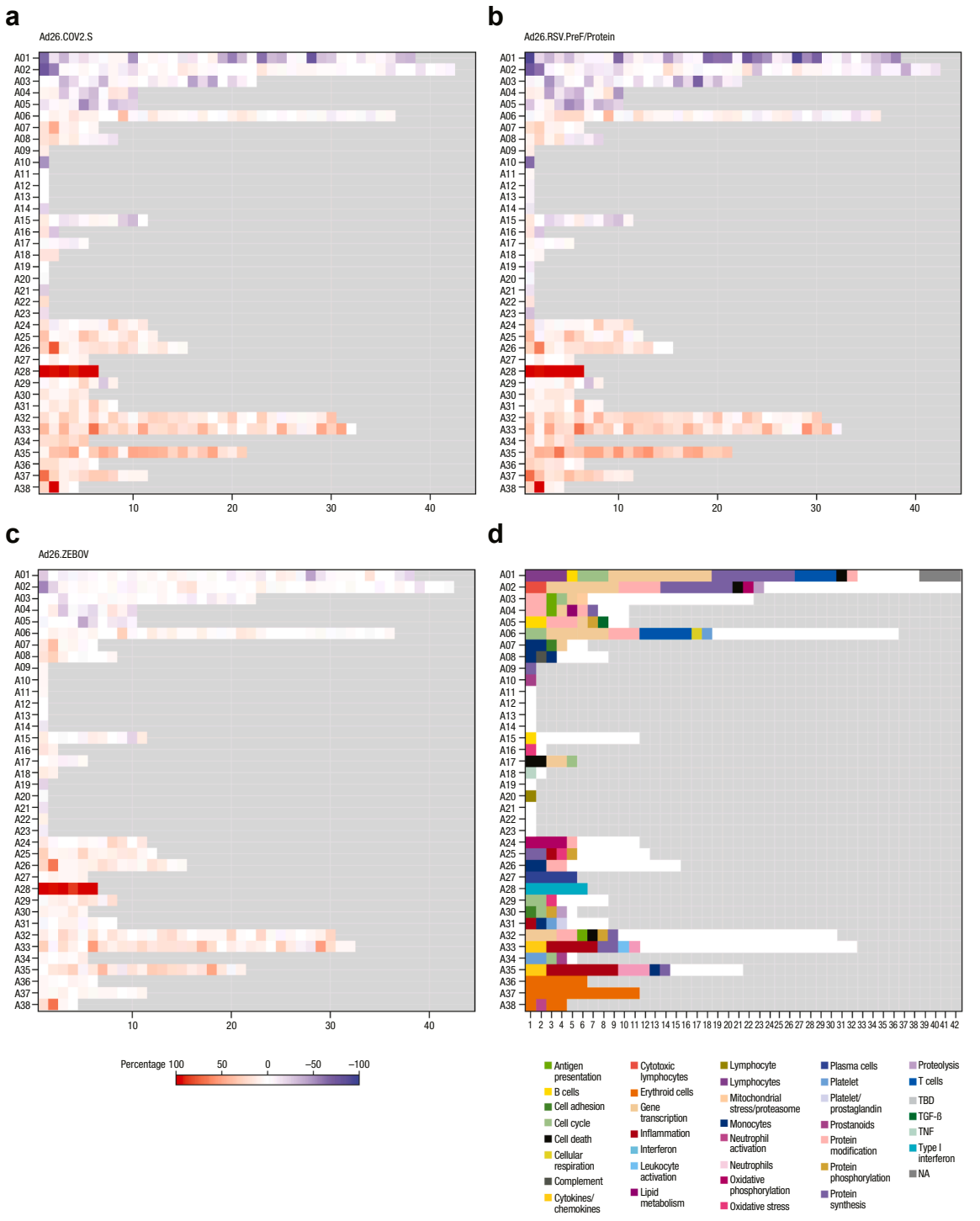

Fig. 5: **Blood transcription module analysis at Day 2 by Ad26 vaccine group.** Heatmaps are shown for a) Ad26.COV2.S, b) Ad.26.RSV.PreF/Protein, and c) Ad26.ZEBOV vaccines. d) shows a fingerprint plot legend with BTM annotations (type/function). A BTM gene set consists of 382 modules covering 14,168 transcripts.[22] These 382 modules were further reduced into 38 individual aggregate modules (gene sets), determined to exhibit coordinated expression across 16 distinct pathological or physiological phenotypes. A gene is considered up- or downregulated when it is significantly differentially expressed ($p < 0{\cdot}05$) and has a >1·5-fold change ($\log_2$ fold change of 0·58). The up- and downregulated modules are then plotted in "fingerprint plots" in which each module has a fixed position. Heatmap colour coding indicates the percentage of genes in the module that are either up- (red) or downregulated (blue). Ad26, adenovirus type 26; BTMs, blood transcription modules; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus.

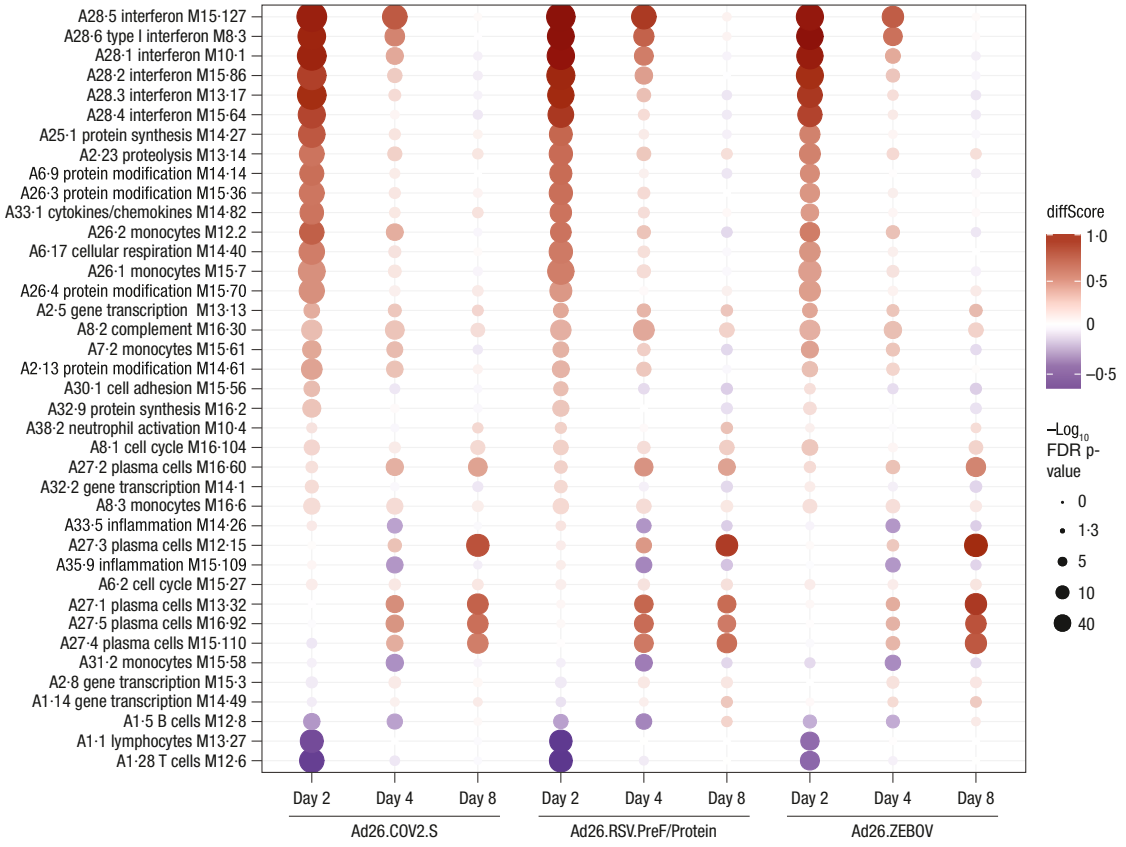

Fig. 6: **An RNA-seq analysis of top differentially expressed blood transcription modules by Ad26 vaccine group and time point.** An independent pathway analysis to support results from the BTM analysis. The difference score ("diffScore") represents the change in expression levels of specific BTMs between different time points from baseline. Positive difference scores indicate upregulation (increased expression; indicated by red) of the gene sets in the BTM, while negative difference scores indicate downregulation (decreased expression; indicated by blue). The dot sizes correspond to the FDR-adjusted p-values. A general type/function of genes is provided, with the leading number indicating the location on the primary BTM legend in Fig. 5d (eg, "A28.5" = Row 28, Column 5). RNA-seq, ribonucleic acid sequencing; BTMs, blood transcription modules; Ad26, adenovirus type 26; preF, prefusion conformation-stabilised RSV F protein; RSV, respiratory syncytial virus; ZEBOV, Zaire ebolavirus.

genes. Several of these modules were also observed to be significantly increased in Cohort 1 following a standard booster dose, albeit with lower absolute levels.

Discussion

Recent studies that have used a systems immunology approach to investigate early vaccine-induced immune responses have been post hoc analyses of multiple datasets, covering transcriptional responses to a variety of vaccines.[3,4,23–25] These studies identified predictive transcriptional signatures of vaccine immunogenicity and reactogenicity, demonstrating that integrating systems biology with clinical and immunological data can unravel vaccine mechanisms and inform tailored vaccination strategies. However, previous studies have not been able to disentangle the role of the insert from that of the vaccine platform. Here, we present findings of a unique study comparing immune responses to three Ad26 vector-based vaccines, in which we conducted comprehensive proteomic and transcriptomic profiling of early responses. The immunogenicity and safety results were consistent with previous studies,[17,26,27] and the presence of preexisting antibodies against the Ad26 vector had no clear impact on insert-specific immunogenicity.[28,29]

All Ad26 vector vaccines were generally well tolerated. The Ad26.RSV.PreF/Protein group had higher rates of AEs than the Ad26.COV2.S and Ad26.ZEBOV groups, which was likely due to the Ad26 RSV group receiving a double dose of the Ad26 vector (1×10^{11} vp vs 5×10^{10} vp), compared to the other groups, and an additional 150 μg of RSV preF protein. Similarly, the innate transcriptional and proteomic responses were also slightly higher in this group. Furthermore, while this Ad26-vectored RSV vaccine is commonly studied in

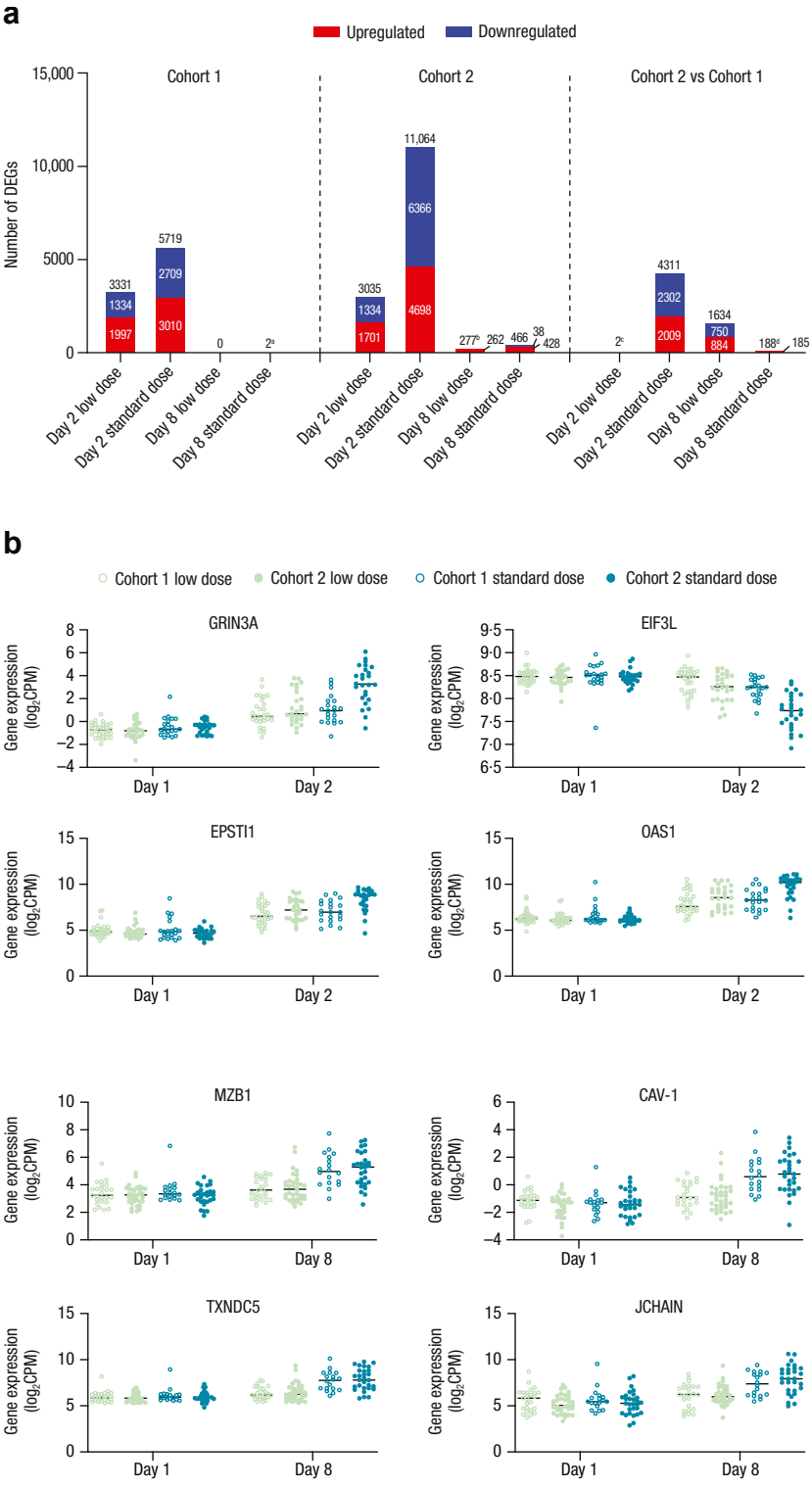

Fig. 7: **Overview of differentially expressed genes by cohort and dose overtime in the COV2008 study.** a) depicts number of DEGs identified (with a >1·5 fold change [absolute log_2 fold change of >0·58]) using a transcriptomic analysis that had an FDR-corrected p-value of <0·05, split by up- and downregulation (red and blue, respectively) across cohorts, booster dose, and time points compared with baseline (prevaccination). b) depicts DEGs across cohorts and booster dose. GRIN3A (part of the glutamate-regulated ion channel superfamily)

adults aged ≥65 years, the higher reactogenicity observed in this small sample size is aligned with a prior study of adults aged 18–59 years.[30] Overall, no new safety signals were encountered, consistent with previous findings.[17,26,27]

Cytokine profiling showed that the strongest responses were observed just 1 day following vaccination and were highest in the Ad26.RSV.PreF/Protein group, followed by Ad26.COV2.S, and lowest in the Ad26.ZEBOV group. Most cytokines upregulated at Day 2 were inflammatory, and cytokine levels were consistently highest in the Ad26.RSV.PreF/Protein group relative to the two other groups, potentially due to the twice as high Ad26 vaccine dose and the inclusion of the RSV preF protein. Of note, the addition of an RSV preF protein did not seem to profoundly change the type of innate response. Similar to our cytokine profiling observations, proteomic analyses demonstrated significant responses at Day 2 across all vaccine groups, which were mostly inflammatory and antiviral in nature. At Days 4 and 8, responses were also related to inflammation and interferons. On Day 8, only TNFRSF8, which is expressed by activated T and B cells and limits autoreactive CD8 T cells, and DUT, which is involved in nucleotide metabolism and interferon-antiviral responses, were primarily upregulated.[31–33] The proteomic data supported the results from cytokine assays (Olink® Target 48), showing that the most dominant vaccine-induced responses occur early on, only 1 day after initial vaccination.

In this study, we showed that the kinetics of the differentially regulated transcriptional immune responses were consistent with the overarching response observed by previous studies, with innate and inflammatory genes being upregulated just 1 day after vaccination and a rise of B cell genes and cellular proliferation after 1 week.[3,34–36] Furthermore, lymphocyte-related gene modules were downregulated at Day 2, which has been observed in transcriptional responses to other vaccines at the same time point and may be due to egress of lymphocytes out of the blood.[3] In response to Ad26 vector-based vaccines, we observed a marked upregulation of interferon-related genes and pathways, supporting results from cytokine assay and proteomic analyses, which demonstrated a strong induction of IFN-γ. In addition, many genes that were upregulated at Day 2, and to a lesser extent at Day 4, were ISGs downstream of type I interferons, indicating that IFN-α and IFN-β were likely produced as well, although these were not directly measured. Because the Ad26 vector is a replication-incompetent viral vector, triggering of antiviral pathways was expected. In a recent study by Blengio et al., responses to the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen showed strong innate signatures at Day 2 and B cell responses at Day 8 after the Ad26.ZEBOV vaccine dose,[37] similar to those observed here. In that study, several genes were correlated with antibody levels, namely HESX1, IFI27, and ATF3, which were also highly upregulated in our datasets at Day 2. At Day 8, many immunoglobulin-heavy variable genes were also significantly upregulated across all three vaccine groups, in addition to Cohort 2 of the COV2008 parallel RNA-seq analysis, two of which were previously described to be correlated with antibody levels.[37] Overall, when directly comparing the responses to the three Ad26 vector-based vaccines, there was no distinct immune signature indicative of an insert-specific response. Rather than differences in DEGs or DEPs between vaccines, differences in magnitude of change were primarily observed, which may have been due to differences in dose. However, a recent study in which two MVA-based vaccines with the SARS-CoV-2 spike protein as insert in differing conformations were compared suggested that the conformation of the vaccine insert could lead to increased immune responses. Therefore, we cannot rule out that the differences in magnitude are due to the immunogenicity of the vaccine inserts.[38]

To further investigate early immune responses to Ad26 vector-based vaccines, a parallel analysis was conducted using participant data from the phase 2 COV2008 study.[10] Participants had received either a single dose of Ad26.COV2.S or two doses of BNT162b2 before receiving a low- or standard-dose Ad26.COV2.S booster. Similar to responses observed in the main study,[10] the strongest immune response was observed 1 day after booster vaccination. However, while responses to the low dose were similar, the standard dose resulted in a greater number of DEGs in the BNT162b2 group compared with those with prior Ad26.COV2.S vaccination. This may be due to de novo B cell induction in the BNT162b2 group, leading to higher anti-spike antibody levels, which was reported in the primary publication of this independent study.[10] Furthermore, prior Ad26.COV2.S exposure might have resulted in anti-Ad26 immunity, dampening responses to an Ad26.COV2.S booster. Although the role of anti-Ad26 immunity was not directly measured, no impact of

functions in physiological and pathological processes in the central nervous system. EIF3L contributes to translation initiation, and enables RNA binding activity. EPSTI1 and OAS1 are related to overall innate responses (downstream of signalling by type I interferons or interferon-γ). CAV-1 (part of the caveolin family) encodes a scaffolding protein that serves as the main component of caveolae plasma membranes. JCHAIN, TXNDC5 and MZB1 are all B cell related genes. DEG, differentially expressed gene; FDR, false discovery rate; CPM, counts per million. [a]Two DEGs upregulated. [b]277 total DEGs, 262 upregulated and 15 downregulated. [c]Two DEGs downregulated. [d]188 total DEGs, 185 upregulated, and three downregulated.

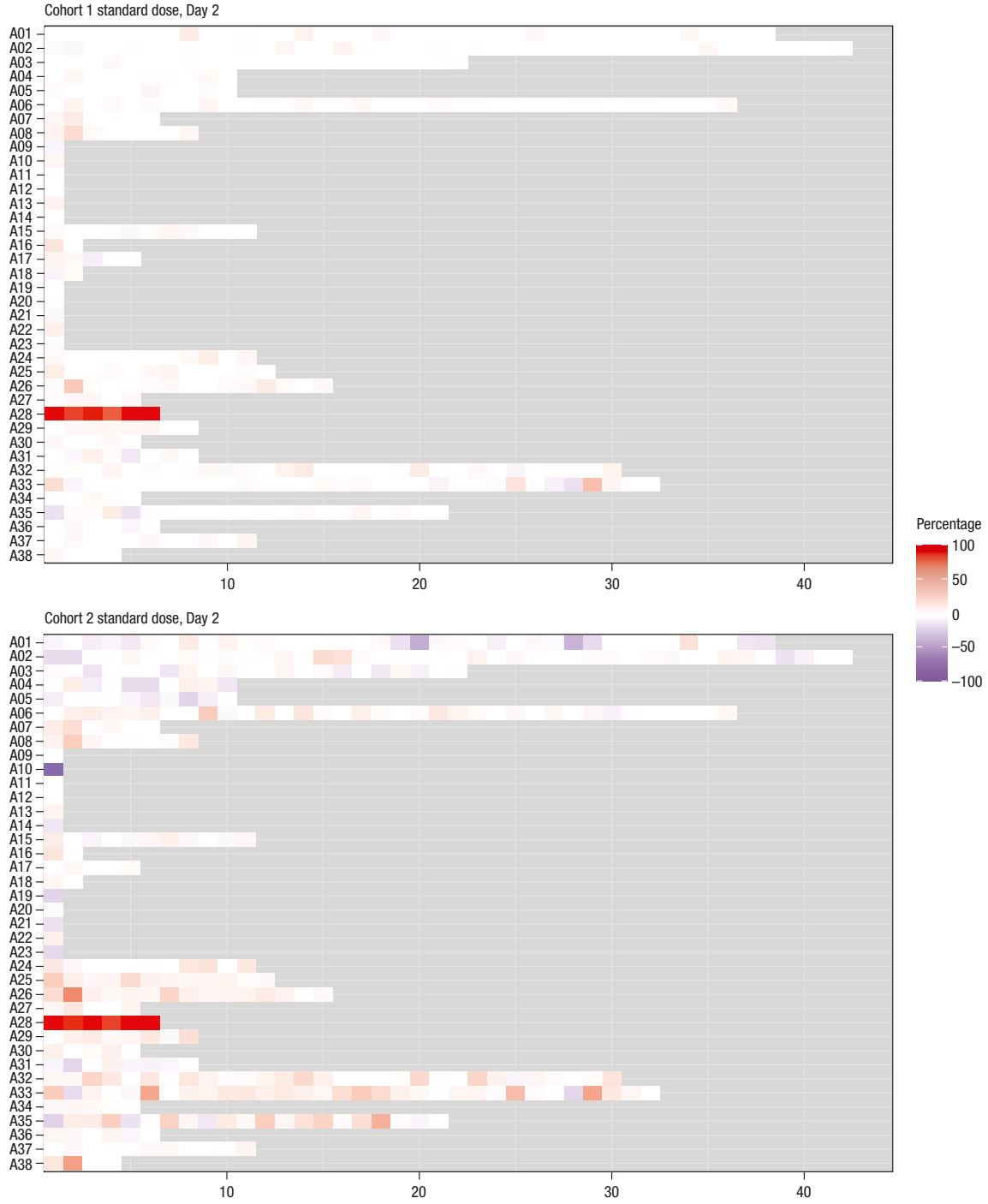

Fig. 8: **Blood transcription module analysis at Day 2 following standard dose booster across cohorts in the COV2008 study.** Heat-maps for each cohort depict either an up- (red) or downregulation (blue) of that module at Day 2, from baseline (before booster vaccination). Respective annotated BTM legend is provided in Fig. 5d. More BTMs were up- or downregulated at Day 2, hallmarked by interferon-stimulated genes (see Row A28). Prior to booster vaccination, Cohort 1 received a single dose of Ad26.COV2.S as primary vaccination and Cohort 2 received two doses of BNT162b2 as primary vaccination. For the Ad26.COV2.S booster, standard dose was defined as 5×10^{10} vp. BTM, blood transcription module.

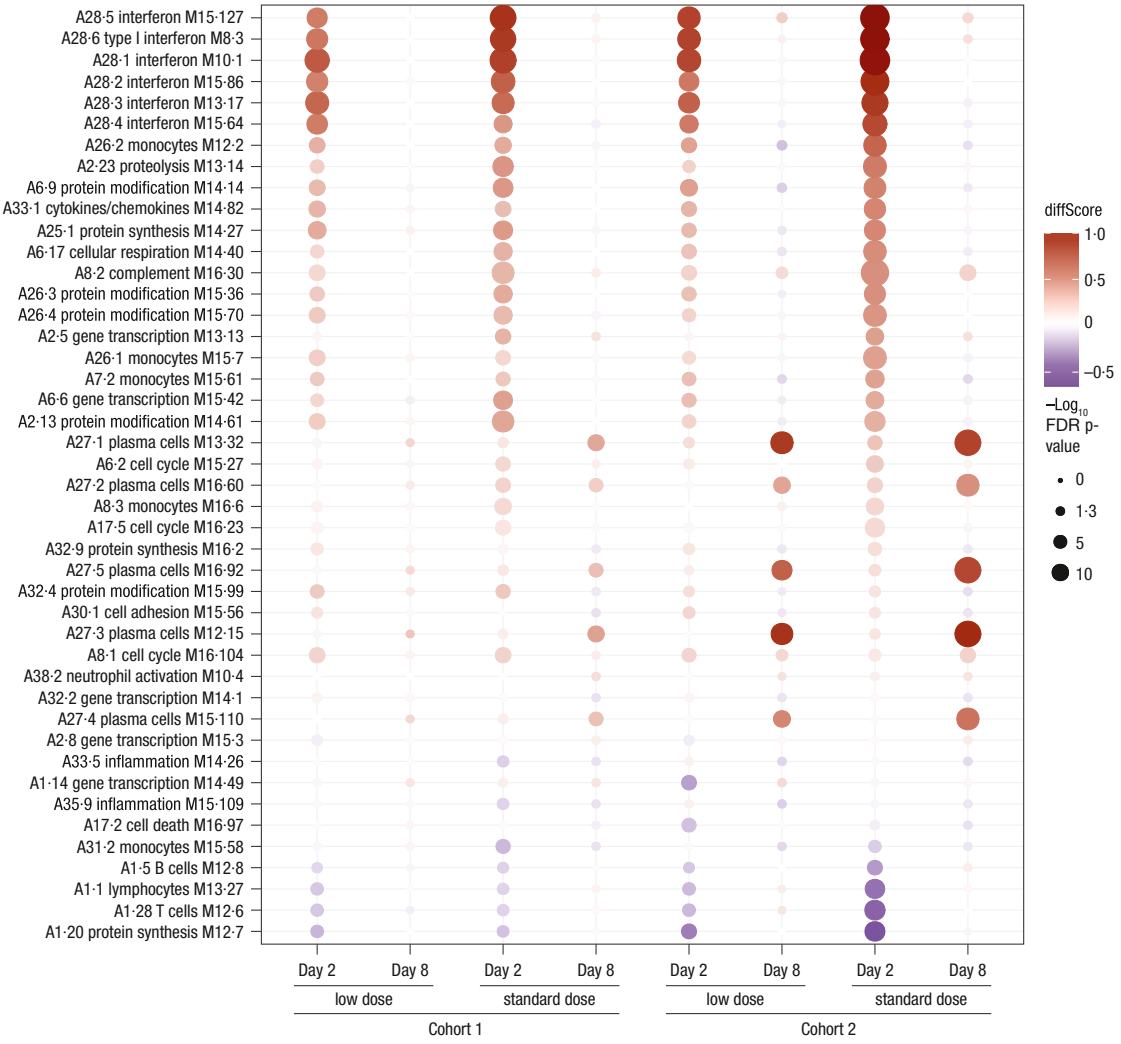

***Fig. 9*: RNA-seq analysis of top differentially expressed blood transcription modules by cohort and dose in the COV2008 study.** An independent pathway analysis to help corroborate results from the BTM analysis (Fig. 8). The difference score ("diffScore") represents the change in expression levels of specific BTMs between different time points from baseline. Positive difference scores indicate upregulation (increased expression; indicated by red) of the gene sets in the BTM, while negative difference scores indicate downregulation (decreased expression; indicated by blue). The dot sizes correspond to the FDR-adjusted p-values. A general type/function of genes is provided, with the leading number indicating the location on the primary BTM legend in Fig. 5d (eg, "A28.5" = Row 28, Column 5). Prior to booster vaccination, Cohort 1 received a single dose of Ad26.COV2.S as primary vaccination and Cohort 2 received two doses of BNT162b2 as primary vaccination. For the Ad26.COV2.S booster, low dose was defined as $1{\cdot}25 \times 10^{10}$ vp and standard dose was defined as 5×10^{10} vp. BTM, blood transcription module; RNA-seq, ribonucleic acid sequencing.

Ad26 nAbs on the booster-induced SARS.COV2.S nAb response was reported in the primary COV2008 study.[10] This observation is consistent with previous studies demonstrating no apparent impact of preexisting Ad26 nAbs on immunogenicity of Ad26-vectored vaccines.[17,29,22]

Our study has several limitations. Since the aim of the study was to compare the established clinical formulations of the three vaccine regimens, the three Ad26 vaccines differed slightly in formulation and dose levels: Ad26.RSV.PreF/Protein was dosed at 1×10^{11} vp and included a protein component, whereas Ad26.COV2.S and Ad26.ZEBOV were both dosed at 5×10^{10} vp, with the Ad26.ZEBOV group also receiving MVA-BN-Filo at Day 57, which was outside of the sampling times for the present study. These differences may have influenced the magnitude of immune response. Additionally, participants had baseline immunity against RSV and SARS-COV-2 but not the Ebola virus. Recruiting participants without preexisting SARS-COV-2 immunity was challenging, limiting our ability to compare immune responses across different

immunity levels. These differences in baseline immunity might have influenced the innate responses. Lastly, the high transcriptional and immunogenicity responses observed in most participants reduced the overall observable differences, preventing correlation analysis.

In conclusion, this is a unique study in humans comparing three vaccines with the same Ad26 vector backbone in a controlled clinical setting, allowing for an in-depth investigation of the impact of the vector and specific inserts on the immune profile. The transcriptomic and proteomic analyses presented here support the findings of the innate mediator profiling, with any differences between the vaccines seemingly related to the magnitude of change, rather than function, of expressed genes and proteins. The dominant factor in the transcriptional and proteomic responses seemed to be the Ad26 vector. These data provide valuable insight and enhance our understanding of the biological mechanisms of action underlying protective innate and adaptive immune responses to Ad26 vector-based vaccines.

Contributors

N.H. contributed to the conceptualisation of the study and the design of the methodology; the coordination for the research activity planning and execution; the data analysis and interpretation; and the writing, review, and editing of the manuscript. N.H. J.S. had access to, and validated the underlying data.

J.S. contributed to the data analysis and interpretation; and the writing, review, and editing of the manuscript. J.S. had access to, and validated the underlying data.

J.T. contributed to the statistical methodology; the data analysis and interpretation; and the writing, review, and editing of the manuscript. J.T. had access to, and validated the underlying data.

R.vH. contributed to the conceptualisation of the study; the protocol design; the oversight and trial execution; data collection; safety data review; and the writing, review, and editing of the manuscript.

N.L. contributed to the conceptualisation of the study; the protocol design; the oversight and trial execution; data collection; safety data review; and the writing, review, and editing of the manuscript.

S.B. contributed to the data analysis; and the writing, reviewing, and editing of the manuscript.

E.M. contributed to the study design, including statistical analysis planning; data analysis and interpretation; and the writing, review, and editing of the manuscript.

M.vR. contributed to data interpretation; and the writing, review, and editing of the manuscript.

L.L. contributed to oversight and trial execution; data collection; safety data review and interpretation; and the writing, review, and editing of the manuscript.

M.M.-L. contributed to the management, planning, and coordination responsibilities for the research activities performed at the external and internal laboratories; and the writing, review, and editing of the manuscript.

C.M. contributed to the study design and methodology; the data analysis and interpretation; and the writing, reviewing, and editing of the manuscript.

K.L. contributed to the conceptualisation of the study; data analysis and interpretation; and the writing, review, and editing of the manuscript.

J.H. contributed to the conceptualisation of the study; data analysis and interpretation; and the writing, review, and editing of the manuscript.

All authors had full access to all data in the study, and accept all responsibility for the submission and publication of the manuscript. All authors read and approved the final version of the manuscript.

The sponsor opted out of including the equitable partnership declaration at this time.

Data sharing statement

The data sharing policy of the Janssen Pharmaceutical Companies of Johnson & Johnson is publicly available at https://www.jnj.com/about-jnj/policies-and-positions/our-position-on-clinical-trial-data-transparency. In accordance with this policy, Johnson & Johnson has established an agreement with the Yale Open Data Access (YODA) Project to act as an independent intermediary for the review and evaluation of requests for access to clinical trial data.

Following publication, de-identified participant-level data and supporting clinical study documentation underlying the results reported in this article may be made available to qualified researchers upon submission and approval of a formal data access request through the YODA Project (http://yoda.yale.edu). Requests must include a clearly defined scientific research proposal and analysis plan, and approved use of the data must be consistent with the scope of analyses permitted under the informed consent provided by study participants.

Access to the data is contingent upon YODA's independent review and approval process and is intended to support research that advances medical knowledge and public health. Additional details regarding the data access process are available via the YODA Project website and the Janssen clinical trial transparency portal at https://www.janssen.com/clinical-trials/transparency.

Declaration of interests

N.H. is a current employee and holds stock options in Johnson & Johnson.

J.S. is a current employee and holds stock options in Johnson & Johnson.

J.T. is a current employee and holds stock options in Johnson & Johnson.

R.vH.is a current employee and hold stocks and stock options in Johnson & Johnson.

N.L. holds stock options in Johnson & Johnson.

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K.L. is a current employee and holds stock options in Johnson & Johnson.

J.H. holds stock options in Johnson & Johnson.

All authors were employees or contractors of Johnson & Johnson at the time the study was conducted.

Acknowledgements

The authors would like to thank Sabrina Spinosa for her contributions to the study, and Harmjan Kuipers, Veronica Rezelj, and Biancamaria Florenzi for their contributions to creating the analysis structure that enabled data analysis. This work was funded by Johnson & Johnson. Medical writing and editorial support were provided by Holly Clarke, PhD, of Lumanity Communications Inc., funded by Johnson & Johnson.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2026.106271.

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