nature medicine

Article

https://doi.org/10.1038/s41591-025-03969-0

Safety and pharmacokinetics of SARS-CoV-2 DNA-encoded monoclonal antibodies in healthy adults: a phase 1 trial

Received: 19 February 2025

Accepted: 18 August 2025

Published online: 21 October 2025

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Local intramuscular administration of synthetic plasmid DNA (pDNA) encoding monoclonal antibodies (mAb) offers an alternative to recombinant protein-based mAb delivery. In this phase 1 dose-escalation study, we evaluated the safety, tolerability and pharmacokinetics of a pDNA cocktail encoding AZD5396 and AZD8076, modified versions of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing mAb cocktail tixagevimab/cilgavimab in healthy adults. Participants received up to four intramuscular doses of pDNA encoding both DNA-based mAbs (DMAbs), administered using CELLECTRA electroporation. The primary endpoints were safety and pharmacokinetics. All 44 participants received at least one dose; DMAbs were detected in 100% of evaluable participants ($n = 39$), with serum concentrations reaching a peak of 1.61 μg ml^{-1}. Sustained expression was observed in all participants during the 72 weeks of follow-up. The study product was well tolerated, with no product-related serious adverse events reported. Exploratory analyses demonstrated binding to multiple SARS-CoV-2 Spike protein variants and neutralizing activity in a standard pseudovirus assay. No antidrug antibodies were detected across approximately 1,000 serum samples using validated tiered assays. To our knowledge, these data represent the first-in-human proof-of-concept that synthetic pDNA DMAb technology permits the durable in vivo production of a functional mAb cocktail. This study further underscores the collective importance of synthetic design, formulation and delivery to achieve biologically relevant expression of gene-encoded biologics. DMAb delivery may represent a long-acting, scalable, cold-chain-independent platform against a wide range of diseases that can be targeted with mAbs and their derivatives. ClinicalTrials.gov registration: NCT05293249

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has driven the global scientific community to advance development of new medical countermeasures. Nucleic acid technologies, including notably mRNA vaccines, had an instrumental role in mitigating the spread of SARS-CoV-2 and reducing COVID-19 disease morbidity and mortality[1,2]. Despite the notable achievements in vaccine development and distribution, a substantial portion of the population remains inadequately protected because of their inability to raise sufficient levels of virus-neutralizing antibodies in response to vaccination[3]. This gap in immunity underscores the need for additional prophylactic and therapeutic approaches for pandemics, which may also be useful as more generalized biological tools.

Infectious disease-targeting monoclonal antibodies (mAbs) have emerged as promising medical countermeasures. Recombinant mAbs have demonstrated the ability to neutralize several strains of

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SARS-CoV-2, thereby offering a potential pathway to both treat[4–8], and prevent COVID-19[9]. Two mAb products received Emergency Use Authorization for the prevention of severe COVID-19 disease and hospitalization in persons who do not respond to vaccination indication: tixagevimab/cilgavimab (authorized from 8 December 2021 to 26 January 2023) and pemivibart (authorized 22 March 2024).

Despite their immense clinical value, the delivery of mAbs as recombinant proteins poses notable delivery hurdles, in addition to manufacturing, logistical and economic challenges. In some cases, intravenous administration can restrict the feasibility of large-scale implementation across broad patient populations. Recombinant mAb delivery also requires a continuous cold chain for stability, presenting further challenges for distribution to resource-limited settings, including low-income and middle-income countries. Optimizing additional strategies to extend the in vivo biological half-life of antibodies and further improve the duration of protection remains a key objective. Innovative solutions are being pursued to improve developmental timelines, delivery and durability of antibody-based interventions. Among these, the use of nucleic-acid-based platforms, such as mRNA, DNA and vectored immune prophylaxis, present new methods for the in vivo production of mAbs. If successful, these techniques could allow a single dose to maintain continuous production of the intended products, making subsequent doses unnecessary.

While in vivo gene-based production of mAbs has generated excitement, challenges have been exposed. These include a short duration of expression for an intravenously delivered mRNA-delivered chikungunya virus mAb[10] and, for studies that achieved longer expression such as adeno-associated virus (AAV)-based vectored immunoprophylaxis, a high incidence (38%, 3 of 8 participants) of antidrug antibody (ADA) induction that prevented continued expression of the mAb transgene[11].

In this study, we evaluated the use of synthetic DNA-based monoclonal antibody (DMAb) technology for the in vivo production of functional antibodies. In response to the COVID-19 pandemic, optimized DMAb versions of AZD5396 and AZD8076 were developed based on the parental monoclonal antibody clones COV2-2130 and COV2-2196, which form the basis of the tixagevimab/cilgavimab cocktail. Preclinical studies in multiple animal models demonstrated robust expression and protective efficacy of this DMAb approach[12,13]. To translate these findings to humans, we conducted a phase 1, dose-escalation trial assessing the safety, tolerability and pharmacokinetics of this DNA-delivered antibody platform in healthy adult participants. In this article, we present full data from the 72 weeks of follow-up, including assessments of in vivo DMAb expression, binding and neutralizing activity, and immunogenicity. These findings support the potential of DNA-based delivery as a long-acting, cold-chain-independent strategy for antibody-based interventions.

Results

Trial design

This phase 1, open-label, single-center, dose-escalation trial evaluated the safety and pharmacokinetic (PK) profile of DNA-encoded mAbs (primary endpoints), with exploratory analyses of antigen binding and virus neutralization, and a post hoc assessment of ADAs; further details on trial design are provided in Methods.

Synthetic DNA plasmids encoding the light and heavy chains (HCs) of AZD5396 and AZD8076 were co-delivered intramuscularly as two separate constructs (pAZD5396 and pAZD8076) for in vivo antibody production. Delivery was facilitated using the CELLECTRA 2000 device with side-port needle to apply localized electroporation (EP) after injection, enhancing muscle uptake and expression efficiency (Fig. 1a,b). The clinical study included eight dosing cohorts with varying injection frequencies, volumes and EP parameters. A schematic overview of the study design and participant flow is shown in Fig. 2; a detailed description is provided in Methods.

Trial participants

Eligible participants were healthy adults aged 18–60 years with a body mass index (BMI) between 20 kg m^{-2} and 30 kg m^{-2}, normal laboratory and electrocardiogram (ECG) findings, and no notable comorbidities or immunosuppressive conditions. Additional inclusion and exclusion criteria are described in Methods and protocol. Sixty-one healthy adults were screened and 44 participants were enrolled and received at least one dose of pAZD5396 and pAZD8076 across eight dosing cohorts (Fig. 2 and Extended Data Table 1). EP was delivered using the CELLECTRA 2000 with OpBlock 0078 for all cohorts except cohort F, which used a modified pulse setting (OpBlock 0070) (Fig. 2a). Sixteen screen failures occurred because of timing ($n = 5$), BMI ($n = 3$), COVID-19 infection ($n = 2$), medical exclusions ($n = 4$) and other factors ($n = 2$). One individual withdrew consent during screening. Among enrolled participants, 61% ($n = 27$) were male and 39% ($n = 17$) were female. Most identified as White (84%, $n = 37$), with additional representation from African American (7%, $n = 3$), Asian (7%, $n = 3$) and Middle Eastern (2%, $n = 1$) ancestries. Baseline demographic characteristics are summarized in Fig. 3a.

Primary endpoints: safety and pharmacokinetics

Product safety. Five participants withdrew early from the study and were replaced per protocol. Four participants discontinued after receiving the first EP dose on day 0; one participant withdrew at week 4 for personal reasons. These withdrawals occurred in cohorts D (one on day 0 and one at week 4), E (one on day 0) and F (two on day 0). One participant in cohort B received only the day 0 dose but agreed to complete the follow-up and was included in the cohort A1 analysis. All participants who received at least one dose were included in the safety analysis. However, the five early withdrawals were excluded from the PK analyses because of insufficient follow-up data. They were replaced as per protocol. The early discontinuations were attributed to discomfort associated with the EP procedure or personal choice and were not related to adverse events (AEs) caused by the study product (Fig. 2a).

As of the prespecified data cutoff (April 2025), 478 AEs were reported among the 44 participants who received at least one dose of the study product. These included 260 related and 218 unrelated events (Supplementary Tables 1 and 2). AEs were categorized into two groups: elicited events, which were systematically collected during the 7 days after each administration, and unsolicited (non-elicited) AEs, which were monitored throughout the study duration.

The most frequently reported elicited events were injection site pain (evaluated using a visual analog scale) and erythema, both of which were transient and typically resolved within minutes of administration (Extended Data Fig. 7). These local reactions are consistent with expected responses to intramuscular injection and EP.

Elicited AEs were further categorized according to severity and body system (Fig. 3b), with comprehensive listings in Supplementary Tables 2, 3, 5 and 8. Detailed characterization is provided in Supplementary Tables 2 and 4, while Supplementary Tables 6 and 7 summarize the number of participants who experienced at least one local or systemic elicited reaction within 7 or 10 days after dosing.

Across all dosing levels, no dose-dependent trend in treatment-related AEs was observed. Most local reactions, including occasional scabbing, were mild and resolved without intervention. Importantly, systemic AEs typically associated with high serum antibody levels were not observed, aligning with the gradual in vivo expression and modest peak DMAb concentrations characteristic of this DNA-based delivery platform. Three serious AEs (SAEs) were reported: two episodes of recurrent pneumothorax in a single participant, one spontaneous miscarriage and a new diagnosis of melanoma in situ that was cured with surgery. All SAEs were assessed as unrelated to study product administration (Supplementary Table 9).

PK profile. Serum concentrations of DMAbs AZD5396 and AZD8076 were measured using validated electrochemiluminescence (ECL)

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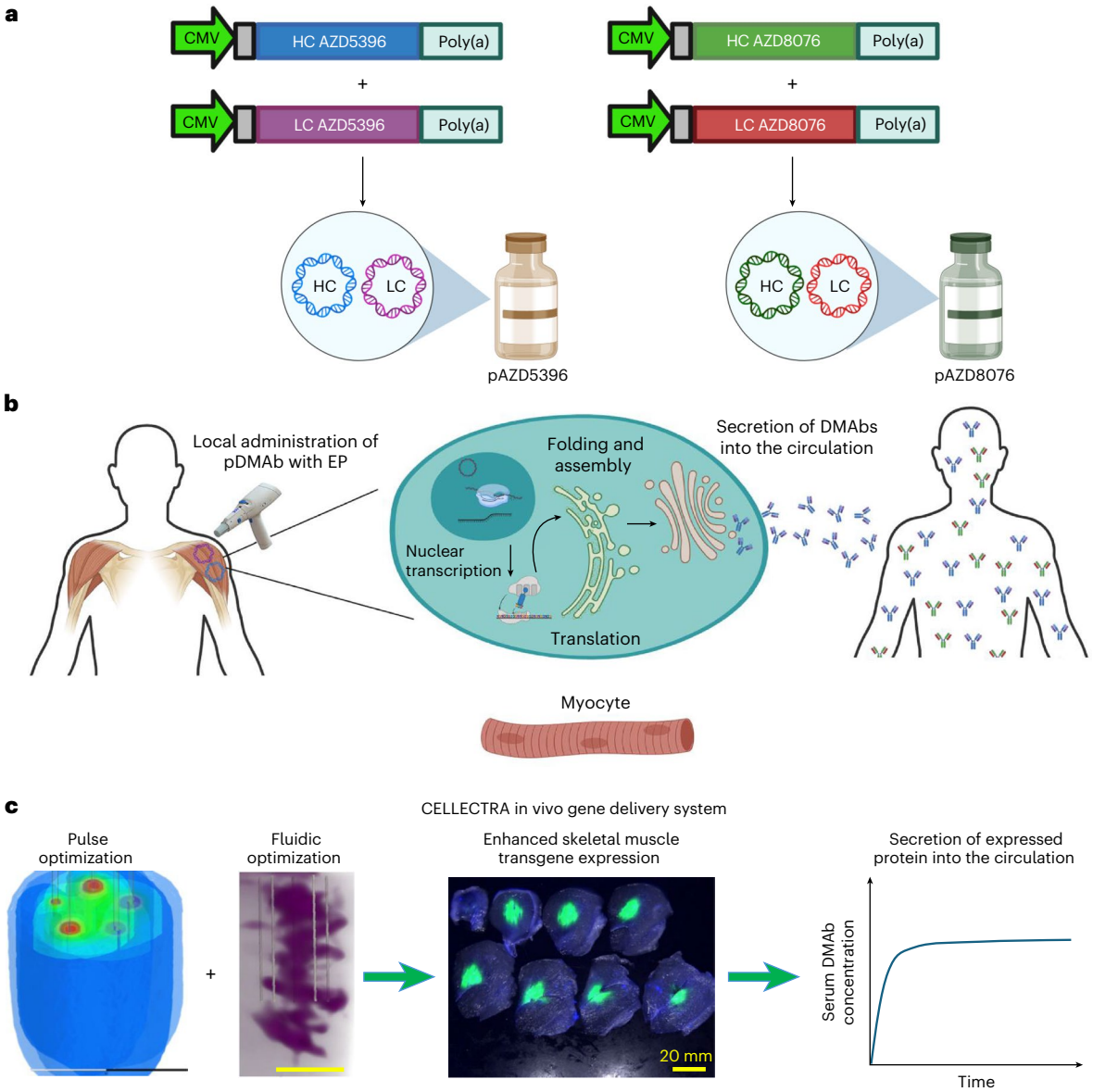

Fig. 1 | Schematic of the DMAb technology platform. a, Four synthetic DNA constructs were designed for optimal in vivo expression of the HCs and light chains (LCs) of AZD5396 and AZD8076. Designs were based on the parental mAb clones COV2-2130 (2130) and COV2-2196 (2196), the precursors of AZD7442, respectively. pAZD5396 and pAZD8076 LC and HC synthetic DNA construct cocktails were each formulated with the human recombinant hyaluronidase enzyme. **b**, pAZD5396 and pAZD8076 were administered separately with in vivo EP for the local expression of the transgenes in the deltoid muscle. DMAbs are expressed in the myocytes and secreted into the circulation. **c**, CELLECTRA EP delivery system for enhanced local transgene expression. The EP electrical field parameters and injected fluid distribution have been optimized to align to permit enhanced transgene expression in the muscle (shown as green fluorescent protein reporter gene expression in New Zealand rabbit muscle). Expressed transgenes, such as DMAbs, can be measured in the serum of the recipient. Illustrations in **a** and **b** created using BioRender.com. CMV, cytomegalovirus.

immunoassays (Supplementary Fig. 1a). Longitudinal PK data were available through week 72 for participants in all cohorts. DMAbs produced in vivo were quantifiable in 100% (39 of 39) of evaluable participants (Fig. 4a–c and Extended Data Fig. 4 (cohort F)). Notably, AZD5396 and AZD8076 were coexpressed at similar levels in individuals, supporting the feasibility of simultaneous delivery of multiple plasmids using this platform (Extended Data Figs. 1 and 2).

The study evaluated how pDNA dose, injection frequency and EP parameters influenced systemic DMAb levels. Escalating the pDNA dose from 0.25 mg (cohort D) to 0.5 mg (cohort B) per injection resulted in an 80% increase in average peak serum concentrations (C_{max}), from 229 to 413 ng ml^{-1} (Extended Data Table 1). However, further increasing the dose to 2.0 mg (cohort E) did not raise the average C_{max}, suggesting saturation at the injection site with current parameters. This trend was supported by the area under the curve (AUC) analysis, which showed comparable serum exposure across cohorts B, C and E, despite increasing doses (Fig. 4f, Extended Data Fig. 3 and Extended Data Table 2).

To evaluate injection frequency, an identical pDNA formulation (0.5 mg per injection) was administered across one-dose (cohort A1), two-dose (cohort B) and four-dose (cohort G) regimens (Fig. 2). Escalating from one to four injections yielded corresponding increases in average C_{max} values, that is, 256 (range 14–379), 413 (range 186–622) and 1,030 (range 646–1,611) ng ml^{-1} (Fig. 4d,e), respectively, reflecting a more than 3.25-fold enhancement in peak DMAb levels (Extended Data Table 2). AUC analyses mirrored these results (Extended Data Fig. 3), supporting a dose-sparing strategy through multisite administration.

Finally, the impact of the EP pulse pattern was assessed by comparing cohort F, which received a truncated delay (0.2 s between

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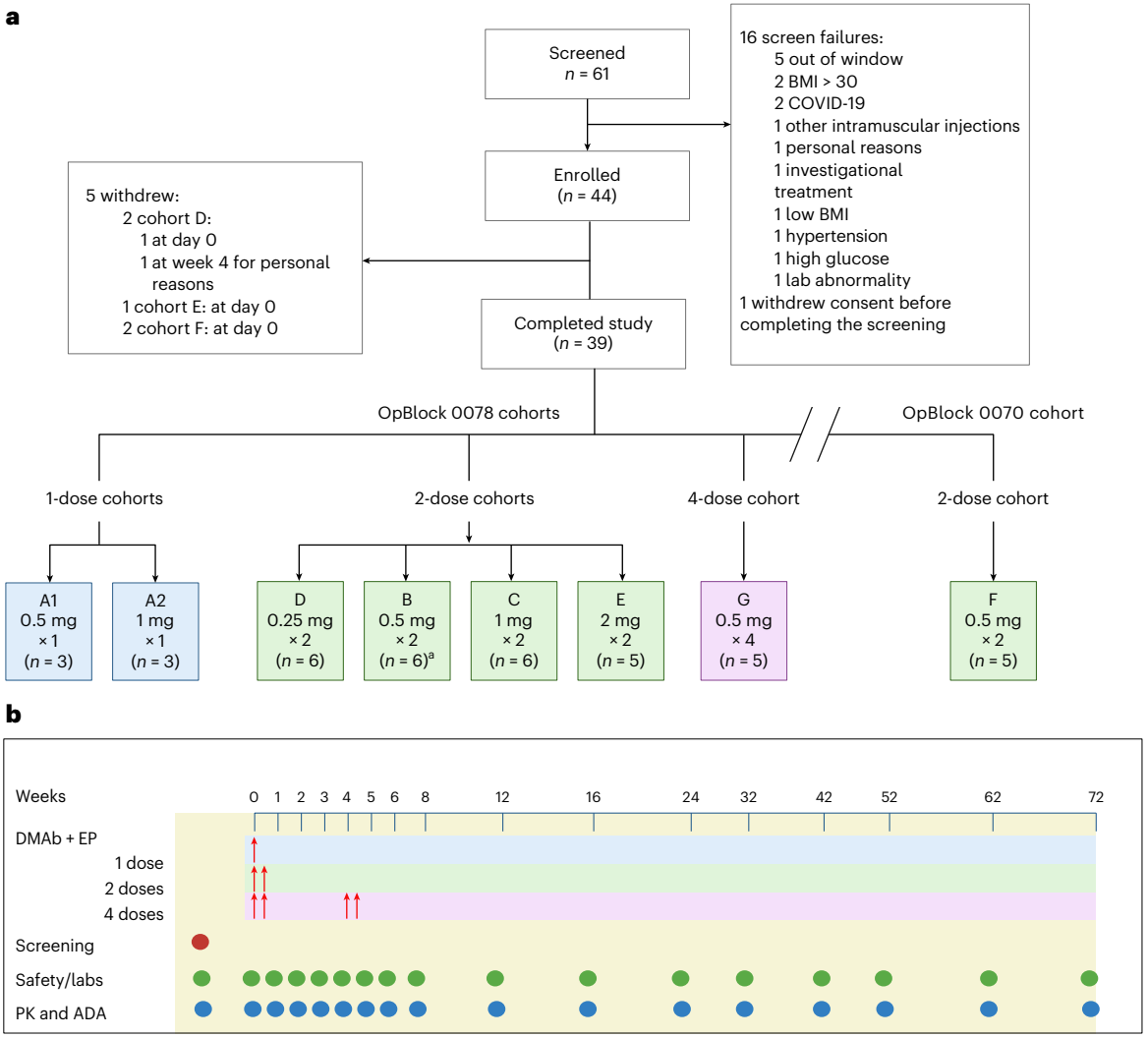

Fig. 2 | Study design, cohort enrollment and participant disposition. **a**, CONSORT-style diagram showing the number of participants screened, enrolled, dosed and included in the safety and PK analyses across all cohorts. [a]One participant in cohort B received only one dose and was analyzed as part of cohort A1. **b**, Diagram of the study design and cohort progression. The trial followed a sequential dose-escalation strategy beginning with two single-dose cohorts: cohort A1 (0.5 mg, single dose) and cohort A2 (1.0 mg, single dose). Subsequent cohorts received multiple doses: cohort B (0.5 mg × 2 doses), cohort C (1.0 mg × 2 doses), cohort D (0.25 mg × 2 doses), cohort E (2.0 mg × 2 doses) and cohort G (0.5 mg × 4 doses; total 2.0 mg). Cohort G assessed repeated multisite injection and cohort F alternative EP parameters (0.5 mg × 2 doses). Enrollment into each cohort was sequential and contingent on safety review of the preceding group. Figure created using BioRender.com.

pulses; OpBlock 0070), with cohort B, which used standard 1-s spacing (OpBlock 0078). Despite using the same plasmid dose and formulation, cohort F achieved similar average peak concentrations (287.9 ng ml^{-1}; range 138–452), although with greater interparticipant variability (Fig. 4 and Extended Data Fig. 4) and worse tolerability, with two participants withdrawing from the study after day 0, which required replacement.

Across all evaluable cohorts, serum DMAb levels increased steadily, peaking around weeks 6–8 after administration. Expression remained stable and durable in all participants for 72 weeks of follow-up.

Exploratory endpoints: DMAb binding and neutralization

The functional activity of DMAbs expressed in vivo was assessed through binding to the SARS-CoV-2 Spike receptor binding domains (RBDs) and pseudovirus neutralization assays. All participants had preexisting anti-Spike antibodies because of prior infection or vaccination (Extended Data Table 3), necessitating use of an anti-YTE-specific antibody to detect AZD5396 and AZD8076 in the serum (Supplementary Fig. 2a). Using this anti-YTE capture, DMAbs were evaluated for RBD binding against several SARS-CoV-2 variants, including ancestral (Wuhan), Delta, Omicron BA.2 and Omicron BA.4/BA.5.

Binding was detected in all 39 participants tested and was sustained through the final available time points, including up to week 72 for participants in cohorts A1–D and up to week 52 for those in cohorts E and F, and up to week 42 for cohort G (Fig. 5 and Extended Data Figs. 5 and 6). Binding levels correlated well with measured serum concentrations from Meso Scale Discovery (MSD) assays (Supplementary Fig. 2b), supporting the long-term stability and biological activity of the DMAbs produced in vivo.

Neutralization activity was tested using a pseudovirus assay targeting ancestral SARS-CoV-2 Spike. To isolate DMAb-specific activity, AZD5396 and AZD8076 were purified from serum using anti-YTE Dynabeads and quantified using both anti-YTE and anti-idiotype enzyme-linked immunosorbent assays (ELISAs) (Supplementary Fig. 3). Neutralizing activity was detected in all 37 participants tested in cohorts A–G, with half maximal inhibitory concentration (IC_{50}) values comparable to those of the parental recombinant antibodies (Fig. 6 and Extended Data Fig. 6). Participants 6302-0014 and

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a

Participant demographics (n = 44)

Variable	Category	Total (n = 44)	Percentage
Sex	Females	17	39%
	Males	27	61%
Race	Asian	3	7%
	Black or African American	3	7%
	Native Hawaiian/Pacific Islander	0	0%
	White	37	84%
	Other (Middle Eastern)	1	2%
Ethnicity	Hispanic or Latino	0	0%
	Not Hispanic or Latino	44	100%
Age	Median (minimum, maximum)	32.5 (20–58)	–

b

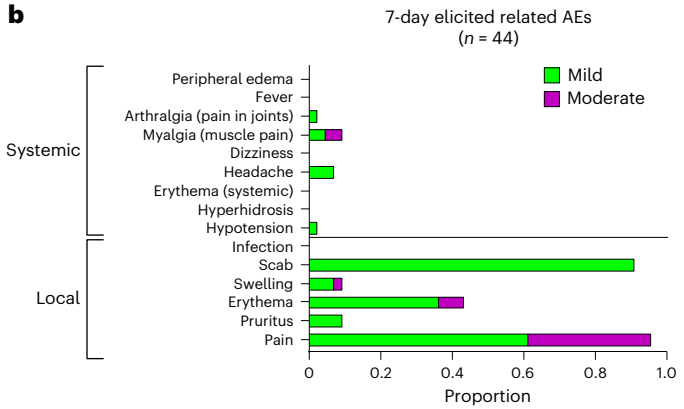

Fig. 3 | Participant demographics and elicited AEs after DMAb administration. **a**, Demographic characteristics of the 44 participants enrolled in the study, including age, sex (self-reported), race and ethnicity. **b**, Frequency and severity of elicited AEs considered related to the study product or administration procedure, reported within 7 days after administration. For each participant, only the most severe grade of each event type is shown. Events are grouped according to category (for example, local, systemic) and graded according to protocol-defined criteria (mild, moderate, severe).

6302-0039 could not be evaluated because of insufficient antibody levels for purification.

Together, these results demonstrate that DMAbs expressed in vivo retained high-affinity binding and neutralization activity against SARS-CoV-2 variants over time.

Post hoc analysis: ADA assessment

To evaluate potential host immune responses against DMAbs expressed in vivo, we used a validated ECL three-tiered ADA assay designed to detect antibodies targeting the Fab region of AZD1061 and AZD8895, and the parental mAbs of AZD5396 and AZD8076, respectively (Supplementary Fig. 1b). Approximately 1,000 serum samples were analyzed from participants who completed dosing.

No confirmed ADAs were detected in any participant. Four individuals (nos. 34, 36, 43 and 58) exhibited positive signals in the tier 1 screening assay for anti-AZD8895 or anti-AZD5396, including one (participant no. 34) with additional screening positivity for both targets. However, all samples tested negative in the tier 2 confirmatory assay and were considered false positives (Fig. 5).

These findings support the immunological tolerability of synthetic DNA-encoded DMAbs, even with multidose and multisite administration. Notably, previous studies of antibody gene delivery using viral vectors demonstrated host immune responses and ADA development that compromised antibody expression[14–16]. In contrast, the absence of ADA detection in this study suggests that the DNA platform may offer a more favorable immunological profile.

Discussion

This study demonstrates the in vivo production of functional mAbs in humans using synthetic DNA delivered via EP. The optimized DNA constructs were formulated to support efficient coexpression of HCs and LCs, with adaptive delivery techniques enabling sustained in vivo expression. Formulation with human recombinant hyaluronidase facilitated diffusion through the extracellular matrix, while side-port needles and the CELLECTRA adaptive EP platform enabled precise, localized delivery. These delivery strategies, developed and validated in animal models, were designed to minimize inflammation and maximize intracellular DNA uptake and expression[12,17,18].

In this phase 1 trial, we evaluated the safety, efficiency and durability of DMAb expression across eight dose-escalating cohorts. Participants received between one and four doses of plasmid DNA (pDNA) encoding AZD5396 and AZD8076. Of the 39 participants who completed the trial, 38 exhibited detectable coexpression of both antibodies throughout the duration of 72 weeks of follow-up, while one expressed only a single mAb. Correct assembly of the antibodies requires nuclear localization, transcription, translation and posttranslational folding and processing through the endoplasmic reticulum and Golgi apparatus. Across 324 plasmid deliveries, successful antibody expression was confirmed in 322 cases, reflecting highly efficient in vivo protein assembly.

DMAb levels were durable and biologically relevant, ranging from hundreds of nanograms per milliliter to over 1 μg ml^{-1} in some individuals. Dose escalation revealed a saturation threshold; increasing pDNA from 0.25 mg to 0.5 mg notably raised expression, but increasing to 2.0 mg did not yield proportional gains. Interestingly, delivering the same total dose across four injection sites resulted in substantially greater expression than delivering it at one site, which is consistent with findings from AAV studies showing spatial limitations to delivery[19].

The DMAbs maintained high-affinity binding to SARS-CoV-2 RBD variants, including the Delta and Omicron sublineages, and demonstrated neutralizing activity in all 37 evaluable participants. This confirms that the antibodies were not only present but also functionally active. Importantly, no ADAs were detected in any of the 39 participants, including those who received multiple doses. This distinguishes the DMAb platform from traditional protein-based mAbs, which are associated with ADA development in 1–10% of cases for fully human products and up to 60% for chimeric or murine antibodies[20].

Recent clinical studies demonstrated the potential of alternative gene delivery platforms for mAb expression. A phase 1 trial of mRNA-1944, which encodes a chikungunya virus-neutralizing antibody, showed dose-dependent expression after intravenous infusion at doses of 0.1, 0.3 or 0.6 mg kg^{-1}. The antibody exhibited a half-life of approximately 69 days, with sustained expression lasting up to 16 weeks at the higher dose levels; however, transient increases in inflammatory markers, such as C-reactive protein, interleukin-6, complement and IP-10, were observed shortly after dosing and resolved within 48 h. Participants in the 0.6 mg kg^{-1} group received corticosteroids to mitigate these inflammatory responses[10].

In parallel, two clinical trials evaluated AAV vectors for in vivo delivery of HIV broadly neutralizing antibodies (bNAbs). In a 2019 study, an AAV1 vector encoding the anti-HIV bNAb PG9 was administered intramuscularly in dose-escalation cohorts. PG9 activity was detected in serum and muscle biopsies in some participants; however, immunogenicity limited the response, with ADA to PG9 and anti-AAV1 immune responses noted, particularly at higher doses[21]. A more recent trial assessed an AAV8 vector encoding the anti-HIV bNAb VRC07. Participants with elevated baseline anti-AAV8 antibody titers were excluded from enrollment. Expression levels of VRC07 exceeded 0.1 μg ml^{-1} in most participants and persisted for up to 150 weeks in one individual. Nonetheless, ADA against VRC07 was observed in three of eight participants, alongside the induction of anti-AAV8 antibodies in most individuals and rare but detectable anti-vector T cell responses[11,14–16].

Our study has several limitations. This analysis was conducted in a demographically homogeneous cohort at a single center. Larger, more diverse studies are needed to evaluate the generalizability of these

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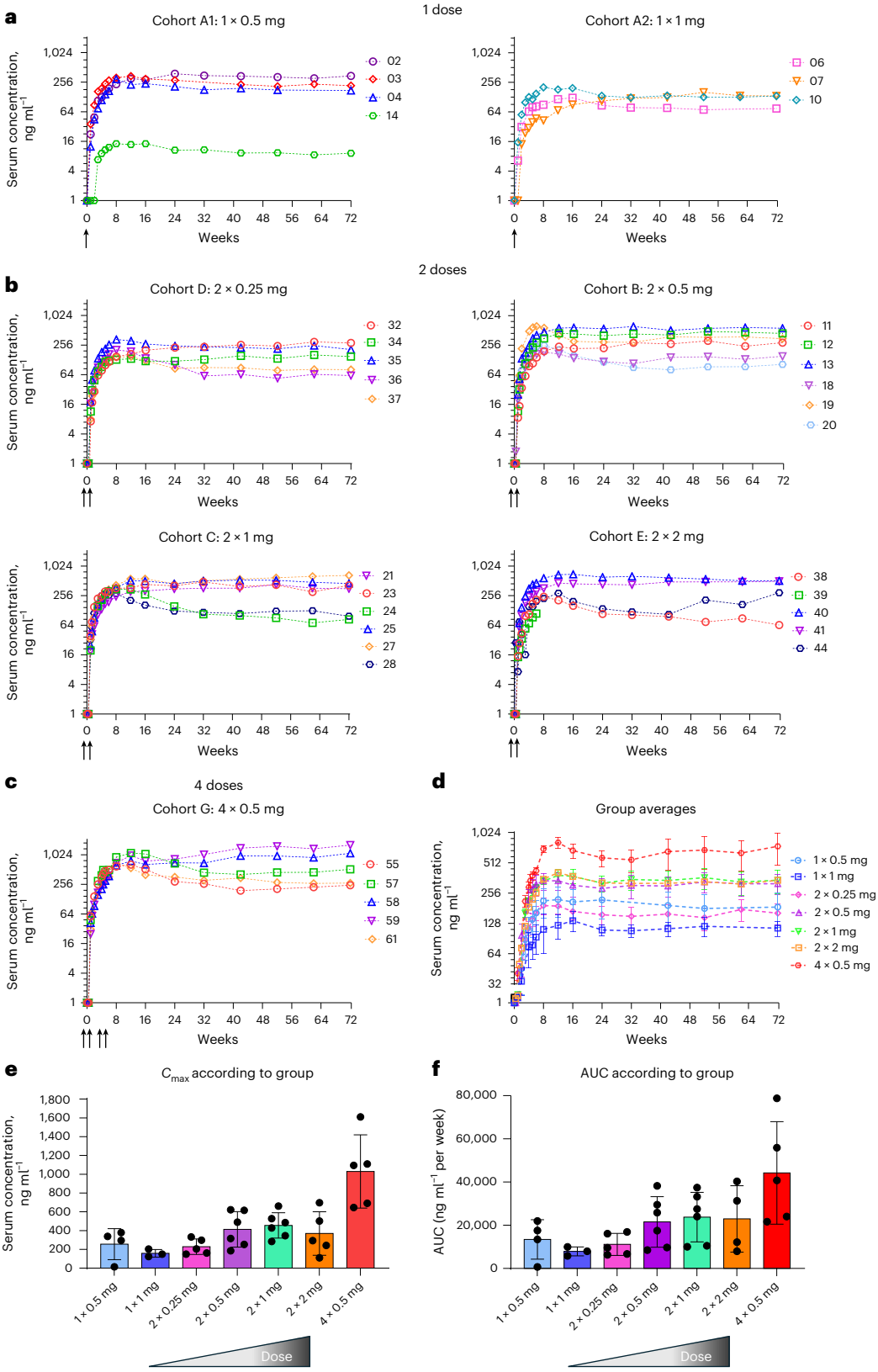

Fig. 4 | Longitudinal serum concentration of the AZD5396 and AZD8076 DMAbs expressed in vivo. a–**c**, Sera collected at the indicated time points after DMAb administration were analyzed using a qualified quantitative binding assay to determine serum concentrations (ng ml^{-1}) in individual participants who received a single dose of 0.5 mg or 1 mg on day 0 (indicated by the arrow) (**a**), two doses of 0.25 mg, 0.5 mg, 1 mg or 2 mg on days 0 and 3 (indicated by the arrows) (**b**) and four doses of 0.5 mg on days 0, 3, 28 and 31 (**c**) (indicated by the arrows). **d**, The mean serum concentrations of dose groups/cohorts are shown. The values shown represent the sum of serum concentrations of the two antibodies. The error bars represent the s.e.m. **e**, Maximum DMAb serum concentration according to group. The bars represent the mean concentrations (±s.e.m.) for each dose group; the points indicate the C_{max} values for individual participants. **f**, AUC through week 72 according to group. The bars represent the mean concentrations (±s.e.m.) for each dose group.

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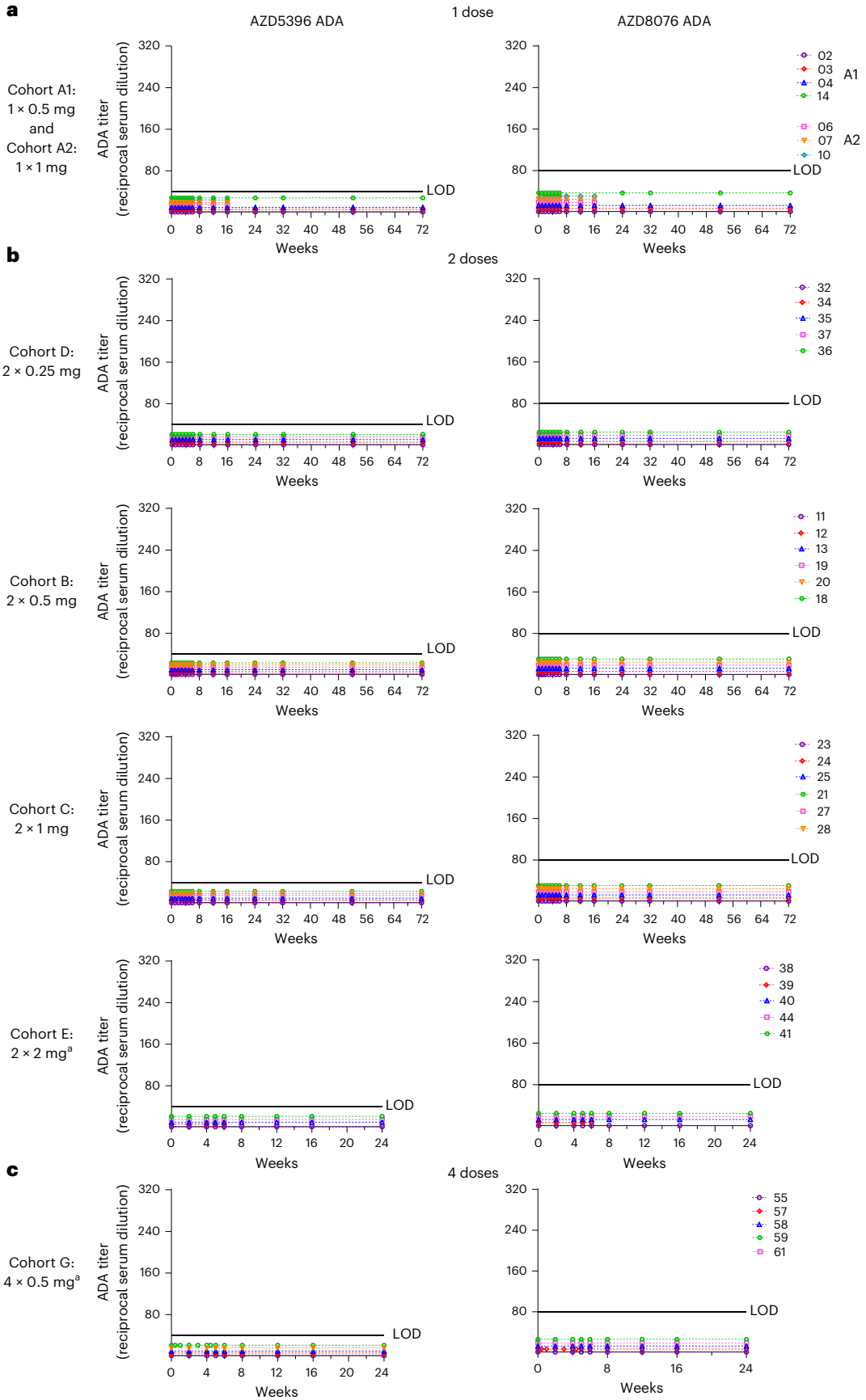

Fig. 5 | Longitudinal measurement of ADA against the AZD5396 and AZD8076 DMAbs. a–c, Anti-AZD5396 (left) and anti-AZD8076 (right) ADA measurements are shown for individual participants in the single-dose cohorts (A1/A2) (**a**), the two-dose cohorts (B–E) (**b**) and the four-dose cohort (G) (**c**). The dotted lines represent the assay minimum titer/limit of detection (LOD) of 40 and 80 for the anti-AZD5396 and anti-AZD8076 assays, respectively. [a]ADA levels were only tested up to week 24, not 72 like in other cohorts.

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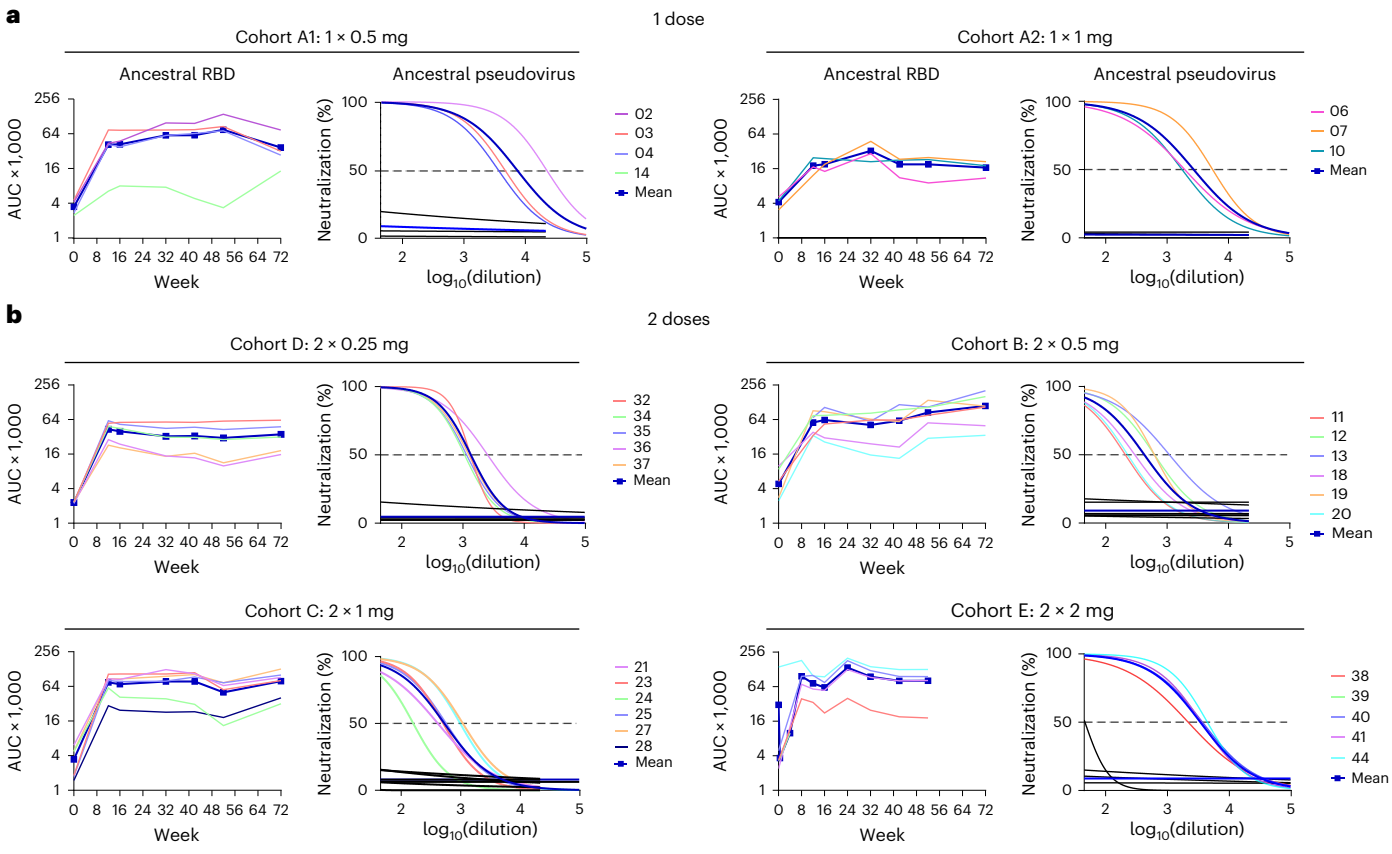

d

Cohort (pDNA dose)			IC_{50} (ng ml^{-1}) (geometric mean, 95% CI)
1 dose	Cohort A1	1 × 0.5 mg	1.21 (0.59–2.50)
	Cohort A2	1 × 1 mg	1.15 (0.32–4.19)
2 doses	Cohort D	2 × 0.25 mg	3.31 (2.64–4.14)
	Cohort B	2 × 0.5 mg	2.20 (1.77–2.76)
	Cohort C	2 × 1 mg	2.13 (1.12–4.06)
	Cohort E	2 × 2 mg	1.61 (0.91–2.85)
4 doses	Cohort G	4 × 0.5 mg	2.34 (1.63–3.36)

Fig. 6 | Longitudinal binding and neutralizing activity of DMAb expressed in vivo against SARS-CoV-2. a–**c**, A DMAb-specific RBD binding assay (left) was developed, in which an anti-YTE antibody was used to capture DMAbs from participant sera followed by probing with biotinylated ancestral Spike RBDs. Graphs are plotted as the AUC × 1,000 for individual participants in each cohort; group means are shown in dark blue. To evaluate antiviral activity, DMAbs were purified from individual participant sera collected either before (day 0) or after (pooled from weeks 12–52) administration using anti-YTE-coated Dynabeads. Purified samples were run in the pseudovirus neutralization assay (right) either unconcentrated or concentrated to be in the range of the assay. The graphs depict the percentage (%) neutralization of individual samples at day 0 (black lines) and after DMAb delivery (colors) when tested at the indicated dilution. Group means are shown in dark blue. RBD binding activity (left) and pseudovirus neutralization (right) are shown for the single-dose cohorts (A1/A2) (**a**), the two-dose cohorts (B–E) (**b**) and the four-dose cohort (G) (**c**). **d**, Average IC_{50} (ng ml^{-1}) of purified DMAbs from the indicated cohorts, calculated as the group geometric mean + 95% confidence interval (CI).

findings and to assess platform performance across varied genetic backgrounds, delivery parameters and clinical settings.

While the levels of mAbs achieved with DMAb administration were below those typically attained immediately after recombinant protein infusion, they remained within the therapeutic ranges reported for multiple biological indications. The observed concentrations persisted for 72 weeks after a single or repeated DNA administration. These findings are promising for long-acting prophylaxis and merit further optimization. Additional formulation development, dose scheduling and EP refinements may increase expression levels and support clinical efficacy.

Importantly, the DMAb levels observed in this study are comparable to those reported for other effective biologics. For example, human anti-chikungunya virus, respiratory syncytial virus and Zika-virus-neutralizing mAbs[22,23] demonstrate functional potency in the low ng ml^{-1} range. Similarly, approved bispecific antibodies such as

Columvi ($C_{trough} \approx 590$ ng ml^{-1})[24] and Blincyto ($C_{ss} \approx 228–537$ pg ml^{-1})[25], which are approved treatments for B cell lymphomas, and Kimmtrak ($C_{ss} \approx 13$ ng ml^{-1}) for treatment of uveal melanoma[26], are active at similar levels. Moreover, protein therapeutics like incretins (for example, GLP-1 analogs) exert biological effects at single-digit ng ml^{-1} concentrations[27]. These precedents support the clinical relevance of the DMAb concentrations achieved in this trial.

Despite its limitations, this study marks a notable advance in antibody gene delivery. We demonstrate that DNA-encoded mAbs can be reliably expressed in vivo with high fidelity, sustained levels and minimal immunogenicity. The consistency of in vivo expression from multivalent plasmid formulations, delivered intramuscularly using optimized EP, suggests robust uptake and transcription within post-mitotic muscle fibers, which are long lived and capable of maintaining episomal DNA without dilution through cell division. This biological context, combined with the method's high take-up rate and codelivery efficiency, may explain the extended antibody expression observed.

This study demonstrates a transformative approach for delivering epigenetic elements without the intention of genetic modification, resulting in long-lived therapeutic potential and circumventing multiple major limitations that have constrained previous strategies. The platform's simplicity, scalability, cold chain independence and potential for cost-effective deployment offer major advantages for global access and equitable distribution. Future studies to expand delivery formats, optimize immune potency and validate protective efficacy in broader populations are warranted. These findings lay the groundwork for a versatile, synthetic, nonviral gene therapy strategy with applications in infectious disease, chronic conditions and beyond.

Online content

Any methods, additional references, Nature Portfolio 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/s41591-025-03969-0.

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Methods

DMAb design and delivery platform

Synthetic DMAb technology was used to enable in vivo production of mAbs targeting SARS-CoV-2. Optimized DMAb constructs were developed in response to the COVID-19 pandemic, encoding AZD5396 and AZD8076, which are based on the parental mAb clones COV2-2130 and COV2-2196, respectively—the precursors of AZD7442. Preclinical studies in mice, hamsters and nonhuman primates demonstrated robust in vivo expression and protective efficacy of this DMAb cocktail[12,13].

Each DMAb was encoded by two synthetic plasmids (pAZD5396 and pAZD8076), encoding the HCs and LCs, respectively. The Fc regions of both antibodies were engineered with the YTE (M252Y/S254T/T256E) mutation to extend the in vivo half-life. Plasmids were administered intramuscularly and delivered using the CELLECTRA 2000 with side-port needle in vivo EP technology[17,18]. The EP system was configured to optimize electric field distribution at the injection site, thereby enhancing plasmid uptake and transgene expression in muscle tissue.

Study design and oversight

This phase 1, open-label, dose-escalation trial was conducted at a single clinical site and approved by the institutional review board (IRB) of the University of Pennsylvania. Enrollment began in May 2022 and was completed in February 2024. All participants provided written informed consent before enrollment.

Eligibility criteria. *Inclusion criteria*.

(1) Age 18–60 years.
(2) Able to provide consent to participate and having signed an informed consent form.
(3) Able and willing to comply with all study procedures.
(4) BMI between 20 and 30, inclusive.
(5) Screening laboratory values within normal limits or with only grade 0–1 findings.
(6) Normal screening ECG or screening ECG with no clinically notable findings.
(7) Women of child-bearing potential who agreed to use medically effective contraception (oral contraception, barrier methods, spermicide) or with a partner who was sterile from enrollment to 6 months after the last injection, or a partner who was medically unable to induce pregnancy. Abstinence was acceptable per investigator discretion if documented and if medically effective contraception was used when engaging in sexual activities, with the study team notified.
(8) Sexually active men considered sexually fertile agreed to use either a barrier method of contraception during the study and for at least 6 months after the last injection, or with a partner permanently sterile or medically unable to become pregnant.
(9) No history of clinically notable immunosuppressive or autoimmune disease. Individuals with HIV infection virologically suppressed for more than 1 year and with current CD4 count greater than 500 cells μl^{-1} were allowed into the study.

Exclusion criteria.

1. Administration of an investigational compound either currently or within 6 months of first dose.
2. Administration of any vaccine within 4 weeks of the first dose.
3. Administration of a SARS-CoV-2 vaccine in the last 90 days or planning to have any standard-of-care vaccines within 14 days from the last administration of the study products.
4. Positive SARS-CoV-2 infection at the screening visit.
5. Administration of any monoclonal or polyclonal antibody product within 4 weeks of the first dose.
6. Administration of any blood product within 3 months of the first dose.
7. Comorbid conditions, including diabetes, hypertension, asthma and any cardiovascular disease.
8. Pregnancy or breastfeeding or planning to become pregnant during the course of the study.
9. Positive serological test for hepatitis B surface antigen or any potentially communicable infectious disease as determined by the Principal Investigator or Medical Director.
10. Positive serological test for hepatitis C (exception: successful treatment with confirmation of sustained virological response).
11. Baseline evidence of kidney disease (creatinine > 1.5 mg dl^{-1}, chronic kidney disease stage II or greater).
12. Baseline screening lab with grade 2 or higher abnormality, except for grade 2 creatinine.
13. Chronic liver disease or cirrhosis.
14. Immunosuppressive illness, including hematological malignancy, history of solid organ or bone marrow transplantation.
15. Current or anticipated concomitant immunosuppressive therapy (inhaled, topical skin/eye corticosteroids, low-dose methotrexate or prednisone <10 mg d^{-1} or equivalent were not exclusionary).
16. Current or anticipated treatment with tumor necrosis factor inhibitors (for example, infliximab, adalimumab, etanercept).
17. Prior major surgery or any radiation therapy within 6 months of the first dose.
18. Any pre-excitation syndromes (for example, Wolff–Parkinson–White syndrome).
19. Presence of a cardiac pacemaker or automatic implantable cardioverter defibrillator.
20. Fewer than two acceptable sites available for intramuscular injection and EP (deltoid and anterolateral quadriceps), including sites with tattoos, keloids, scars within 2 cm of the injection site; implantable cardioverter defibrillator/pacemaker ipsilateral to the deltoid site (unless deemed acceptable by a cardiologist); or metal implants/medical devices at the EP site.
21. Prisoner or participants who are compulsorily detained for treatment of a physical or psychiatric illness.
22. Active drug or alcohol use or dependence that would interfere with adherence to the study requirements or assessment of immunological endpoints.
23. Not willing to allow storage and future use of samples for SARS-CoV-2 virus-related research.
24. Any illness or condition that, in the opinion of the investigator, may affect participant safety or endpoint evaluation.
25. Known bleeding diathesis or use of blood thinners within 30 days before enrollment (low-dose aspirin (81 mg daily) acceptable).
26. Concomitant intramuscular medications.
27. Known previous intolerance or contraindication to methylprednisolone (for participants in cohort D).

Participants were recruited through the University of Pennsylvania Clinical Trials Unit using IRB-approved advertisements, outreach to prior research volunteers and investigator referrals. As enrollment was voluntary, potential self-selection bias is acknowledged; however, this is unlikely to affect the internal validity of the study given its primary objectives of assessing safety and pharmacokinetics in healthy adults.

Study design. Initially designed as a five-cohort dose-escalation study, the protocol underwent multiple amendments in response to U.S. Food and Drug Administration (FDA) and IRB feedback, as well as emerging clinical and procedural data. The protocol was also temporarily amended to allow use of the ProFusion therapeutic needle after concerns about the original needle, which were subsequently resolved.

Cohort D was revised to receive a reduced dose without methylprednisolone. As the study progressed, three additional cohorts (E, F and G) were added, expanding the total number to eight. Additional follow-up time points and flexibility in PK/ADA sampling were introduced to improve PK modeling. Based on the tolerability data, EP parameters were changed from OpBlock 0070 to OpBlock 0078 for all cohorts except cohort F.

Participants received intramuscular injections of synthetic DNA plasmids formulated with HYLENEX Recombinant to enhance tissue permeability and improve the efficiency of in vivo transfection, in line with earlier reports[17,18]. The study followed a single ascending dose design using a modified 3 + 3 schema across eight cohorts (A1–G), with plasmid doses ranging from 0.25 mg to 2 mg and injection volumes from 0.25 to 1 ml. HYLENEX doses ranged from 34 to 135 units, adjusted in proportion to the plasmid dose and volume. Some cohorts received a single injection on day 0, while others followed a more intensive schedule with additional doses on days 3, 28 and 31 (see Fig. 2 and Extended Data Table 1 for cohort-specific details).

Sixty-one healthy adults were screened; of these, 44 participants received at least one dose of the investigational DNA plasmids pAZD5396 or pAZD8076. Sixteen individuals did not meet the eligibility criteria because of timing constraints ($n = 5$), BMI ineligibility ($n = 3$), active COVID-19 infection ($n = 2$), other medical exclusions ($n = 4$) or personal or external reasons ($n = 2$). One participant withdrew consent before completing screening (Fig. 2, top). Study oversight was provided by an independent Data and Safety Monitoring Board (DSMB).

Sequential enrollment began with cohort A1, where the first participant in each new cohort was dosed and monitored for 14 days before dosing the remaining participants. This waiting period was waived for cohorts D, F and G, where doses were comparable to or lower than those previously tested. Because of the higher dose administered in cohort E, despite lower expression levels observed in earlier cohorts compared to protein-based mAbs, the 14-day observation period was reinstated. In all cohorts, subsequent participants were dosed 3 days apart. If dose-limiting toxicity (DLT) occurred in the first three participants of a cohort, the DSMB conducted an ad hoc review to determine whether to proceed. If deemed unsafe, the remaining participants would have not been enrolled, although the next cohort would not open until 28 days of safety monitoring had been completed. Any dose level with more than one DLT among six participants would have been considered not tolerated. No DLT was observed in the trial.

Each participant underwent a comprehensive baseline evaluation upon consent, including demographic and medical history, concomitant medication review and assessment of eligibility. EP was performed using the CELLECTRA 2000 device. All cohorts were treated using the OpBlock 0078 parameter set, except for cohort F, which used OpBlock 0070. Dosing strategy, plasmid formulation, HYLENEX concentration and the EP settings for each cohort are summarized in Fig. 2.

Safety assessments

Safety was assessed in all participants who received at least one dose of the investigational product ($n = 44$). Participants were monitored for AEs using the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials[28], with laboratory abnormalities evaluated based on site-specific reference ranges. Until the reporting cutoff date, AEs have been documented, including the severity of all related and unrelated AEs (systemic and local) by body system, descriptions of those AEs, elicited local reactions for the first 7 and 10 days after dosing, and the number of individuals who experienced at least one elicited local or systemic reaction in the first 7 days after dosing.

Clinical safety evaluations included vital signs, physical examinations, 12-lead ECG, complete blood counts with differential, serum chemistries, HbA1c, coagulation parameters, serologies, creatine phosphokinase, urinalysis, pregnancy testing (when applicable), PK sampling, ADA assessments and a nasopharyngeal swab for SARS-CoV-2 (Fig. 2b).

The primary safety evaluation focused on the occurrence, nature, timing, duration, intensity and relatedness of both injection site and systemic AEs elicited within 7 days of each administration. Events were categorized using the Medical Dictionary for Regulatory Activities by System Organ Class and Preferred Term. SAEs were collected throughout the study and characterized according to onset, outcome and relatedness to the investigational product. Injection site pain was assessed using a visual analog scale immediately, and at 5 and 10 min after injection/EP, after each dose.

Injection sites (deltoid or quadriceps) varied according to participant preference. Local and systemic elicited AEs were actively monitored for 7 days after each dose. Laboratory safety and PK assessments were conducted at screening, day 0 and day 7, and weeks 2, 3, 4, 5, 6, 8, 12, 16, 24, 52 and 72 after injection. Additional labs were obtained on days 3 and 10 in the two-dose cohorts, and on days 28 and 31 in the four-dose cohorts. All anti-AZD5396 (clone M5B4.2E9) and anti-AZD8076 (clone M16H5.1) AEs, including injection site reactions, were monitored through week 72.

If a predefined safety stopping criterion was met, the study would be paused for review by the DSMB and study team. This did not occur. All safety data were summarized descriptively, with particular attention to potential dose-related trends.

Antibodies

The following antibodies were used in binding ELISAs, pseudovirus neutralization assays and PK/ADA assays.

Commercial antibodies.

- Horseradish peroxidase (HRP)-conjugated goat anti-human IgG (polyclonal) (1:8,000 dilution, cat. no. 2049-05, Southern Biotech).
- HRP-conjugated streptavidin (1:10,000 dilution, cat. no. N100, Thermo Fisher Scientific).

AstraZeneca research-grade antibodies.

- Anti-YTE monoclonal antibody (clone 23F7.1) used at 1–2 μg ml^{-1}.
- Anti-idiotype mABs anti-AZD5396 (clone M5B4.2E9) and anti-AZD8076 (clone M16H5), used at 1 μg ml^{-1}.

Anti-YTE mAb (clone 23F7.1), anti-idiotype clone M5B4-2E9 and anti-idiotype clone M16H5.1 were provided by AstraZeneca and have been described previously[13]. All primary reagents were confirmed using ELISAs. The anti-YTE mAb demonstrated specific binding to YTE-containing AZD5396 and AZD8076 but not to control human IgG serum. Anti-idiotype clone M5B4-2E9 binds specifically to AZD5396; clone M16H5.1 binds specifically to AZD8076. For all ELISAs, recombinantly purified anti-AZD5396 and anti-AZD8076 antibodies were used as standards to confirm reagent specificity.

All antibodies were validated either by the manufacturer, in prior peer-reviewed publications, or in-house during assay development. Full validation details and supporting references are provided in Reporting Summary.

Quantification of AZD5396 and AZD8076 in serum

AZD5396 and AZD8076 were measured using separate ECL bridging immunoassays. For quantitation of AZD5396, 50 μl each of biotinylated anti-idiotypic antibody and SULFO-TAG (ruthenium)-labeled anti-YTE antibody (each at 1 μg ml^{-1}) were mixed in a 96-well polypropylene plate with 50 μl of study serum samples pre-diluted 1:5 in MSD Diluent 100 (Meso Scale Discovery). Plates were incubated on a shaker at 600 rpm for 1 h. In parallel, MSD Streptavidin Gold plates were blocked with 200 μl per well of StartingBlock blocking buffer (Thermo Fisher Scientific) for at least 30 min, then washed three times with PBS-Tween 20.

Then, 100 µl of the reaction mix was transferred to the streptavidin plate and incubated for 30 min at 600 rpm. After three washes with PBS-Tween 20, 150 µl of 2× MSD Read Buffer T was added, and the plate was read on an MSD Sector S600 Imager. AZD5396 concentrations were interpolated from a standard curve using recombinant AZD1061 protein (range 1.23–300 ng ml^{-1} in 20% pooled normal human serum).

The assay for AZD8076 followed the same procedure, except that biotinylated anti-YTE and SULFO-TAG-labeled anti-idiotypic antibodies for AZD8076 were used at 0.25 µg ml^{-1} each. The standard curve used recombinant AZD8895, which is identical in amino acid sequence to AZD8076 except for the YTE substitutions also present in AZD1061.

Detection of ADAs against AZD5396 and AZD8076

Study serum samples were assessed for the presence of ADAs using a validated three-tiered ECL assay (screening, confirmation and titer), previously validated by PPD for tixagevimab (AZD8895) and cilgavimab (AZD1061), as described in ref. 29. AZD5396 and AZD8076 share identical Fab sequences with their parental mAbs AZD1061 and AZD8895, respectively. Unlike the Fc-silenced versions used in the original AZD7442 product, the DMAbs in this study retained their Fc effector function, bringing the expressed antibodies structurally and functionally closer to native human IgG1. Therefore, the validated ADA assay, which targets the Fab region, is appropriate for detecting immune responses against the expressed DMAbs. The Fc regions also include YTE mutations to extend the half-life, which have not been associated with notable ADA induction in prior clinical studies. For samples with signals below the cutoff points in screening or confirmatory assays, ADA titers were reported as less than 40 for AZD5396 and less than 80 for AZD8076, reflecting the minimum required serum dilution for the respective assays.

Anti-SARS-CoV-2 Spike protein RBD endpoint titer ELISA

Preexisting antibodies against SARS-CoV-2 at study entry (day 0) were evaluated using an ELISA; 96-well plates were coated overnight at 4 °C with 1 µg ml^{-1} SARS-CoV-2 RBD proteins from ancestral (cat. no. 40592-V08H, Sino Biological), BA.4/5 (cat. no. 40592-V08H130, Sino Biological) and JN.1 (cat. no. 40592-V08H155, Sino Biological) strains. The following day, plates were washed with PBS + 0.05% Tween 20, blocked with Blocker Casein in PBS (cat. no. 37528, Thermo Fisher Scientific) for 90 min at room temperature, then incubated for 1 h with participant sera serially diluted fourfold in blocking buffer. After washing, HRP-conjugated goat anti-human IgG (1:8,000 dilution) was added for 30 min at room temperature. Plates were developed with 1-step Turbo TMB (cat. no. 34022, Thermo Fisher Scientific), stopped with 2N H_2SO_4 and read at 450/570 nm using a BioTek Synergy Neo2 plate reader (Agilent Technologies). Endpoint titers were determined using the method by Frey et al.[30] and analyzed using Excel and Prism v.10.2.1 (GraphPad Software).

Detection of DMAbs expressed in vivo binding to SARS-CoV-2 RBD

Because all participants had preexisting anti-Spike RBD antibodies, a modified capture ELISA was performed to detect DMAbs produced in vivo; 96-well flat-bottom half-area plates (Corning) were coated overnight at 4 °C with 2 µg ml^{-1} anti-YTE mAb (clone 23F7.1). Plates were washed with PBS-Tween 20 and blocked with 5% nonfat dry milk + 0.2% Tween 20 in PBS for 1 h at room temperature. Sera were diluted 1:4 and serially in twofold steps in 1% newborn calf serum with 0.2% Tween 20 in PBS, and incubated on the coated plates for 2 h at room temperature. Biotinylated SARS-CoV-2 RBD proteins representing wild-type (cat. no. 793906, BioLegend), Delta, BA.2 (cat. no. SPD-C82Eq, ACRO Biosystems) and BA.4/BA.5 (cat. no. SPD-C82EW, ACRO Biosystems) variants were used for detection. After incubation, plates were washed and incubated with HRP-conjugated streptavidin (1:10,000, cat. no. N100, Thermo Fisher Scientific) developed with Ultra TMB (cat. no. 34028, Thermo Fisher Scientific), quenched with 2N H_2SO_4 and read at 450/570 nm on a BioTek Synergy 2 plate reader. Data were exported to Excel, analyzed using Prism v.10 and reported as AUC over time.

Pseudovirus neutralization assay

HEK 293T cells transformed with SV40 large T antigen (CRL-3216, ATCC) were used for producing the SARS-CoV-2 Spike pseudovirus. Cells were maintained according to the supplier's recommendations in DMEM supplemented with 10% FCS and 1% penicillin-streptomycin. All experiments involving cell lines were conducted under approved institutional biosafety protocols.

Pseudoviruses bearing the SARS-CoV-2 Spike protein (USA-WA1/2020 isolate) were produced by cotransfecting HEK 293T cells with a 1:1 ratio of pNL4-3.Luc.R-E- plasmid (NIH AIDS Reagent Program) and various Spike-expressing plasmids (GenScript) using GeneJammer (Agilent Technologies). After 48 h, the viral supernatants were collected and supplemented with FCS to a final concentration of 12%.

huCHOAce2 cells (cat. no. VCeL-Wyb019, Creative Biolabs) were plated at 10,000 cells per well in 96-well plates in D10 medium (DMEM + 10% FCS + penicillin-streptomycin), then incubated overnight. The next day, heat-inactivated serum or purified DMAb samples were serially diluted and incubated with pseudovirus for 90 min at room temperature before transfer to huCHOAce2 cells. Plates were incubated for 72 h, then lysed using britelite plus (cat. no. 6066769, Revvity); luminescence was read using a BioTek Synergy 2 plate reader. Neutralization curves were fitted using Prism v.10 (nonlinear regression with Hill slope <0). Minimal infective dose (ID_{50}) values were calculated as the reciprocal dilution yielding 50% neutralization. IC_{50} values were derived by dividing the serum DMAb titer by the ID_{50}.

Purification of DMAbs from participant sera

DMAbs were purified from serum using anti-YTE-coated Dynabeads (MyOne Tosylactivated, cat. no. 65501, Thermo Fisher Scientific). Day 0 or pooled week 24–52 sera were diluted 1:4 in PBS and incubated with anti-YTE Dynabeads overnight at 37 °C. Bead complexes were washed and eluted using Pierce IgG Elution Buffer (cat. no. 21004, Thermo Fisher Scientific). Two elution fractions were collected. For cohorts A1, A2 and D, samples were concentrated using Amicon 30 kDa MWCO (Merck) filters. DMAb concentration was confirmed using ELISA with anti-YTE capture and secondary anti-idiotype mAbs (M54-2E9 for AZD5396, M16H5.1 for AZD8076). Plates were coated overnight with 1 µg ml^{-1} anti-YTE or 5 µg ml^{-1} anti-idiotype mAbs, washed, developed using the same protocol as RBD ELISAs and read on a BioTek Synergy Neo2 reader. Data were analyzed in Gen5 and graphed using Prism v.10.2.1.

Statistical analyses

This phase 1 study was not powered for hypothesis testing. Cohort sizes were determined based on prespecified DLT monitoring parameters, consistent with FDA guidance for first-in-human clinical trials. Participants were enrolled in cohorts of $n = 3$, 5 or 6 depending on dose level, with a target DLT threshold of 30% used to guide cohort expansion or modification.

In addition, smaller sample sizes in the extension phase of the study (cohorts E–G) were primarily determined by the available resources. The goal was to maximize the scientific information obtained, particularly regarding the impact of multisite administration and EP parameters, within the constraints of limited funding and manufacturing capacity. These exploratory cohorts were designed to generate informative PK and safety data to guide future development, rather than to formally test predefined hypotheses.

Statistical analyses were performed using descriptive methods. AEs were tabulated according to dose level, system organ class and preferred term, with frequencies, percentages and exact 90%

Clopper–Pearson CIs calculated. Laboratory values, vital signs and other continuous measures were summarized using the mean, s.d., median and range, while PK endpoints, including detection rates and time to a 50% decline from peak concentration, were estimated with corresponding CIs. Disposition, including enrollment, dose administration, study completion and discontinuations, was also described. Interim analyses were conducted in response to emerging safety events; missing data were managed using participant replacement per investigator discretion. No formal power analysis was performed because the study focused on estimation rather than hypothesis testing. All statistical analyses were performed using STATA v.16 (StataCorp). Prism v.10.0 was used for data analysis and graph generation.

Reporting summary

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

Data availability

All requests for raw and analyzed clinical trial data and materials will be promptly reviewed by the corresponding authors (P.T. and D.B.W.) to determine if they are subject to intellectual property or confidentiality obligations, particularly regarding planned PK modeling analyses to be reported in a subsequent publication. Any data and materials that can be shared will be released via a material transfer agreement (requested from D.B.W. and The Wistar Institute, for review by The Wistar Legal Group). Sequences for the plasmids pAZD5396 and pAZD8076 have been submitted under patent no. WO2023064841A1. Plasmids can be shared on completion of a material transfer agreement with the corresponding authors.

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Acknowledgements

We thank The Wistar Institute Core facilities. This work was funded by the Defense Advanced Research Projects Agency and the Joint Program Executive Office (JPEO) for Chemical, Biological, Radiological and Nuclear Defense (CBRND) (award no. HR0011-21-9-0001 to D.B.W.) and reagents and assay support from 4P01AI165066-02 for this effort. This project was supported by the JPEO-CBRND Joint Project Lead for Enabling Biotechnologies in collaboration with the Defense Health Agency COVID funding initiative for the phase 1, dose-escalation trial of the safety and pharmacokinetics of SARS-CoV-2 DMAb in healthy adults, The Wistar Institute, Inovio Pharmaceuticals, AstraZeneca and the University of Pennsylvania (HR0011-21-9-0001) for this effort. We also recognize support from P01AI165066-02 (D.B.W. and J.P.), and NIH/NIAID Collaborative Influenza Vaccine Innovation Centers (CIVIC) contract 75N93019C00051 (D.B.W., J.P. and A.P.) for reagents and assay tools. The views expressed in this press release, poster and manuscript reflect the views of the authors and do not necessarily reflect the position of the Department of the Army, Department of Defense nor the United States Government. References to nonfederal entities do not constitute or imply Department of Defense or Department of the Army endorsement of any company or organization. Funding sources were not involved in the design of this study, the collection and analyses of the data, the decision to submit or the preparation of the manuscript. We thank the participants and their families and the following DSMB members: I. Lee, M. Schuster and K. Bar.

Author contributions

P.T., A.P., E.M.P., L.H., T.R.F.S. and D.B.W. designed the study. M.C. served as the regulatory liaison and supported the FDA submissions and communications. A.B. managed the clinical study team. C.T. contributed to participant recruitment and follow-up. K.M. provided data management and statistical support. The other authors developed and validated several assays. A.P. and E.M.P. also contributed critical technical insight to assay design and interpretation. The first draft of the manuscript was written by P.T., D.B.W., A.P. and E.M.P. All authors contributed to data interpretation, reviewed the manuscript and approved the final version for submission.

Competing interests

D.B.W. has received grant funding, participates in industry collaborations, has received speaking honoraria and has received fees for consulting, including serving on scientific review committees and board series. Remuneration received by D.B.W. includes direct payments and stock or stock options. D.B.W. also discloses the following paid associations with commercial partners: Geneos (advisory board), AstraZeneca (advisory board, speaker), Inovio (BOD, SRA, stock) and Sanofi (advisory board). J.A., D.A., P.F., L.H. and T.R.F.S. are employees of Inovio Pharmaceuticals; as such, they receive salary and benefits, including ownership of stock and stock options. P.E.L., J.R.F. and M.T.E. are employees of and hold or may hold stock in AstraZeneca. A.P., E.M.P., T.R.F.S, D.W.K. and D.B.W are listed as inventors on patent application no. WO2023064841A1 'Antibodies for use against SARS-COV2', published 20 April 2023, filed by The Wistar Institute. This application includes the HC and LC plasmid sequences for pAZD8076 HC, pAZD8076 LC, pAZD5036 HC and pAZD5036 LC described in this manuscript. Plasmids are available upon request and completion of a material transfer agreement. The other authors declare no competing interests.

Additional information

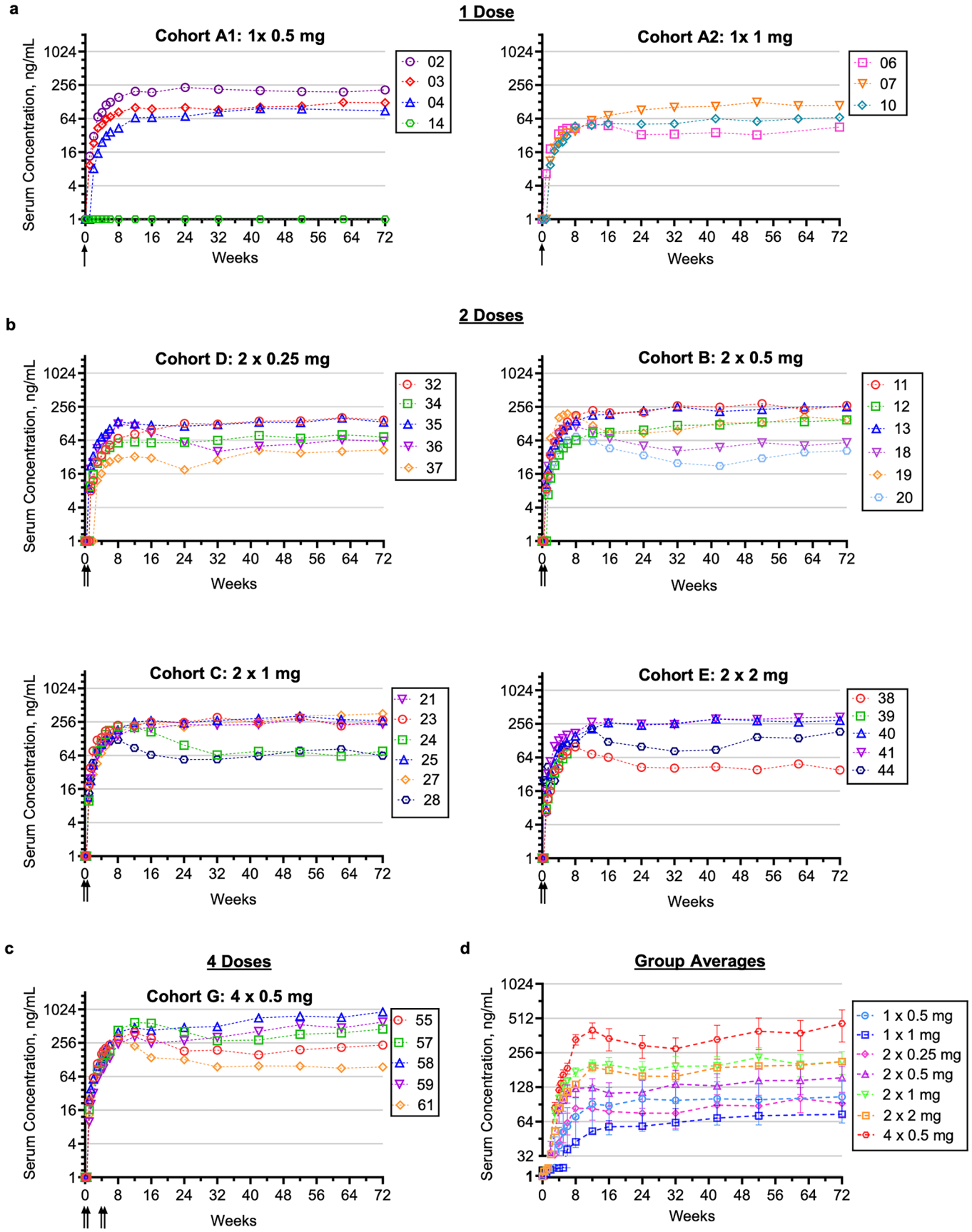
Extended data is available for this paper at https://doi.org/10.1038/s41591-025-03969-0.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-025-03969-0.

Correspondence and requests for materials should be addressed to Pablo Tebas or David B. Weiner.

Peer review information *Nature Medicine* thanks Benjamin Goldman-Israelow, James Kublin and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Saheli Sadanand, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-025-03969-0



Extended Data Fig. 1 | Longitudinal serum concentration of *in vivo*-expressed DMAb AZD5396. Serum DMAb concentrations (ng/mL) measured using a qualified quantitative binding assay are shown for study participants receiving **a**, a single dose of 0.5 mg or 1 mg on D0; **b**, two doses of 0.25 mg, 0.5 mg, 1 mg, or 2 mg on D0, 3; or **c**, four doses of 0.5 mg of SARS-CoV-2 DMAb on D0, 3, 28, 31. Arrows indicate dosing events. **d**, Mean serum concentrations (±SEM) for each dose group.

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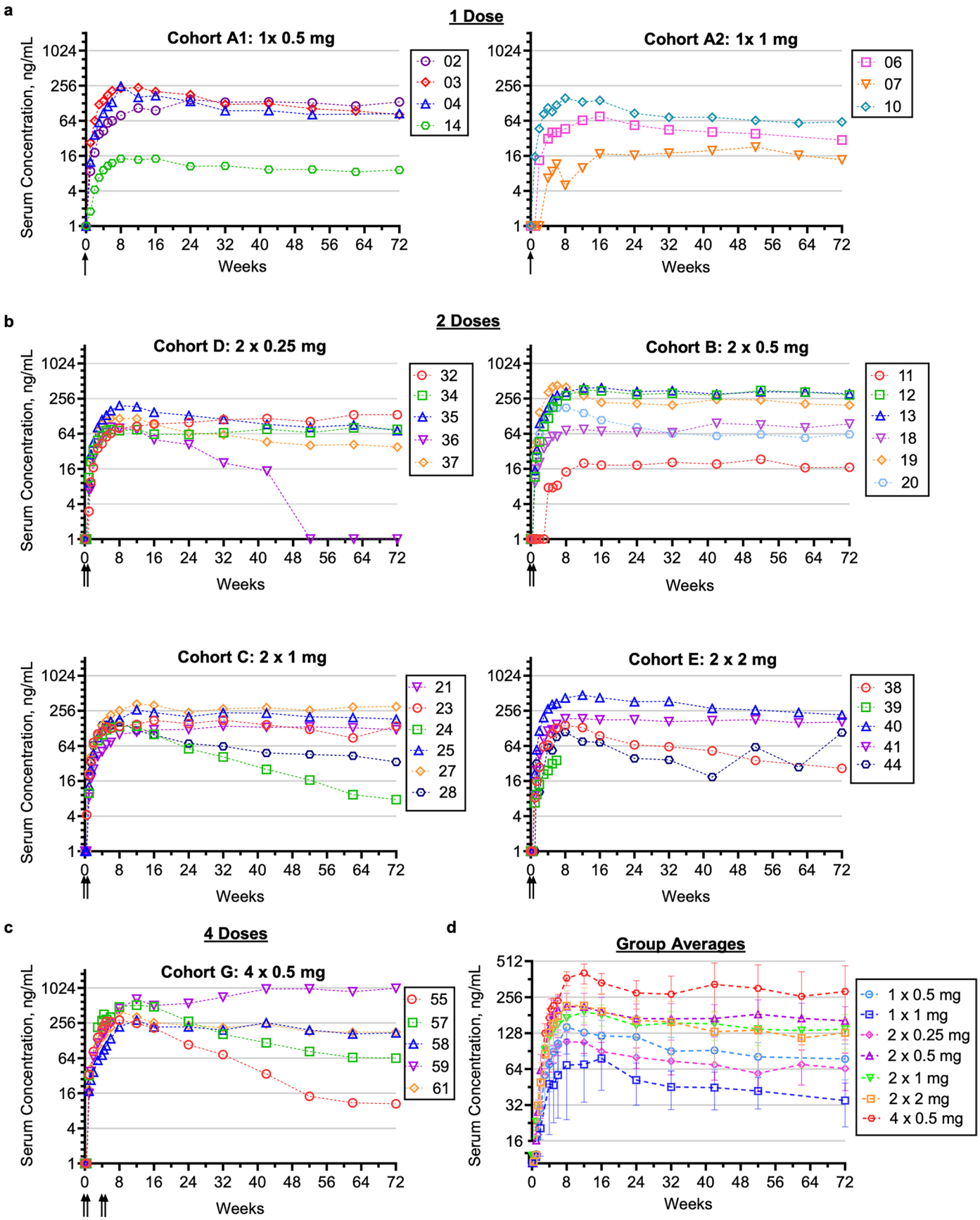
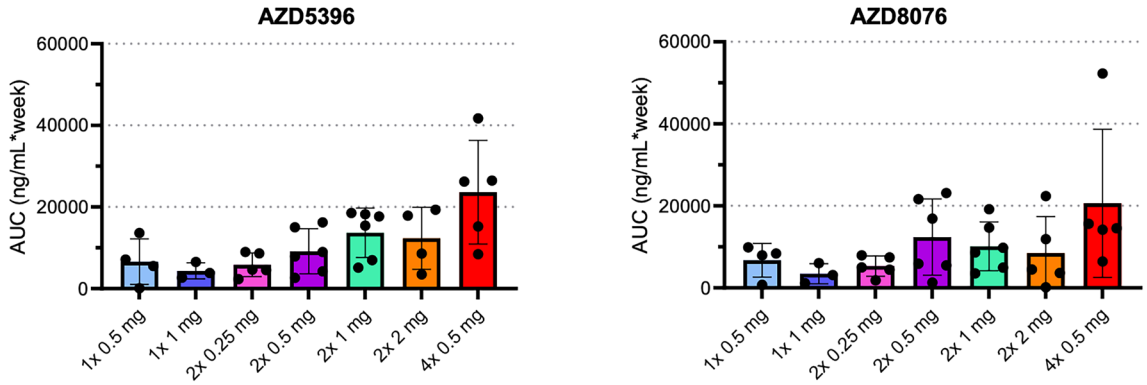


Extended Data Fig. 2 | Longitudinal serum concentration of *in vivo*-expressed DMAb AZD8076. Serum DMAb concentrations (ng/mL) measured using a qualified quantitative binding assay are shown for study participants receiving **a**, a single dose of 0.5 mg or 1 mg on D0; **b**, two doses of 0.25 mg, 0.5 mg, 1 mg, or 2 mg on D0, 3; or **c**, four doses of 0.5 mg of SARS-CoV-2 DMAb on D0, 3, 28, 31. Arrows indicate dosing events. **d**, Mean serum concentrations (±SEM) for each dose group.

https://doi.org/10.1038/s41591-025-03969-0



Extended Data Fig. 3 | Area under the serum SARS-COV-2 DMAb concentration-time curve for indicated dose groups after 24 weeks from the initial dose. AUC data are shown for AZD5396 (left), AZD8076 (middle), and total DMAb (right). Bars represent mean concentrations (±SEM) for each dose group. Points indicate AUC values for individual participants.

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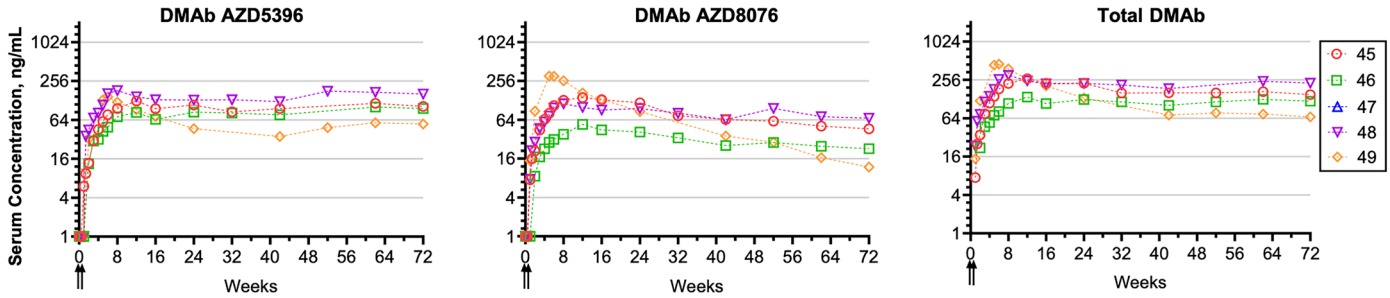
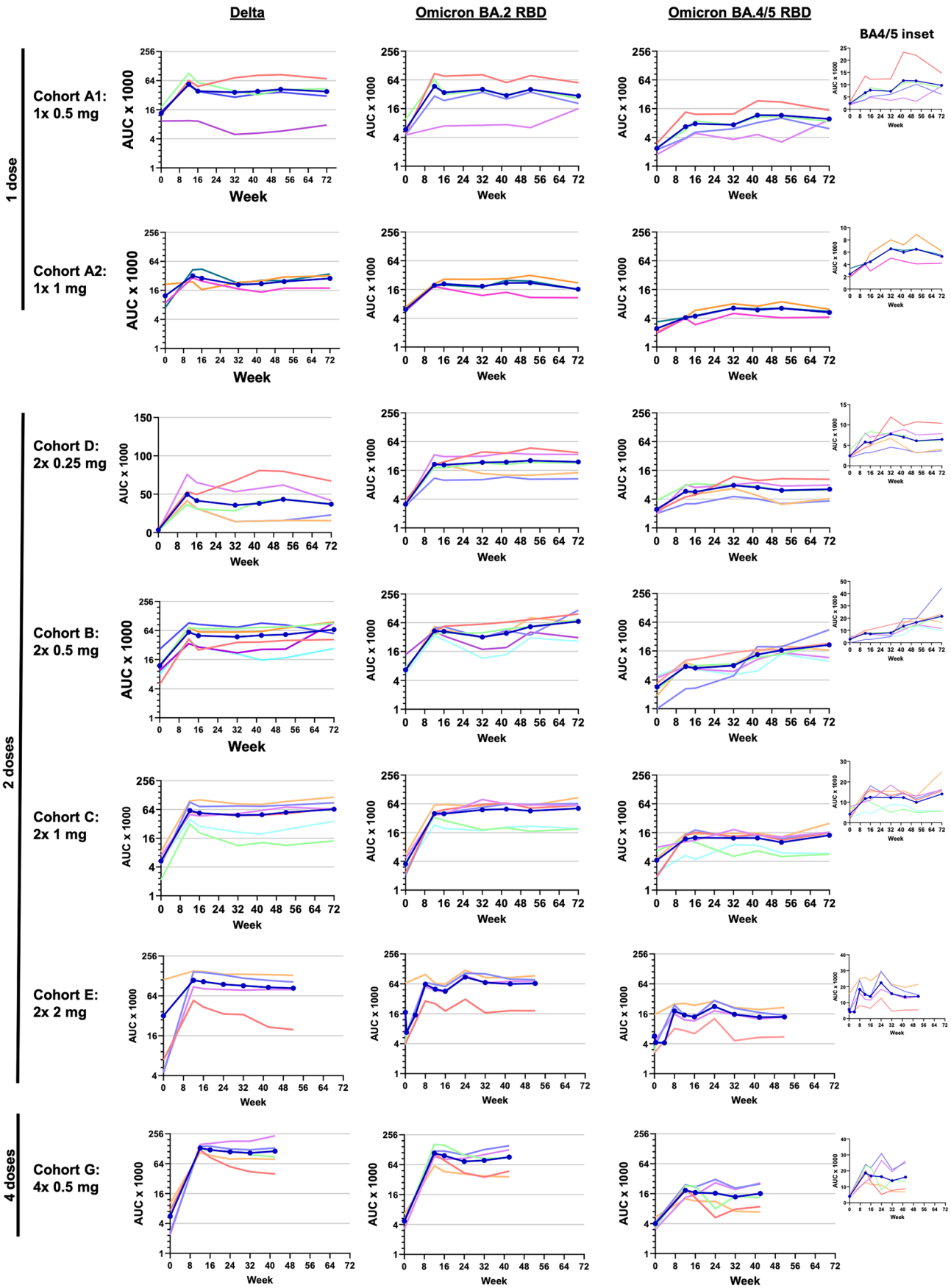


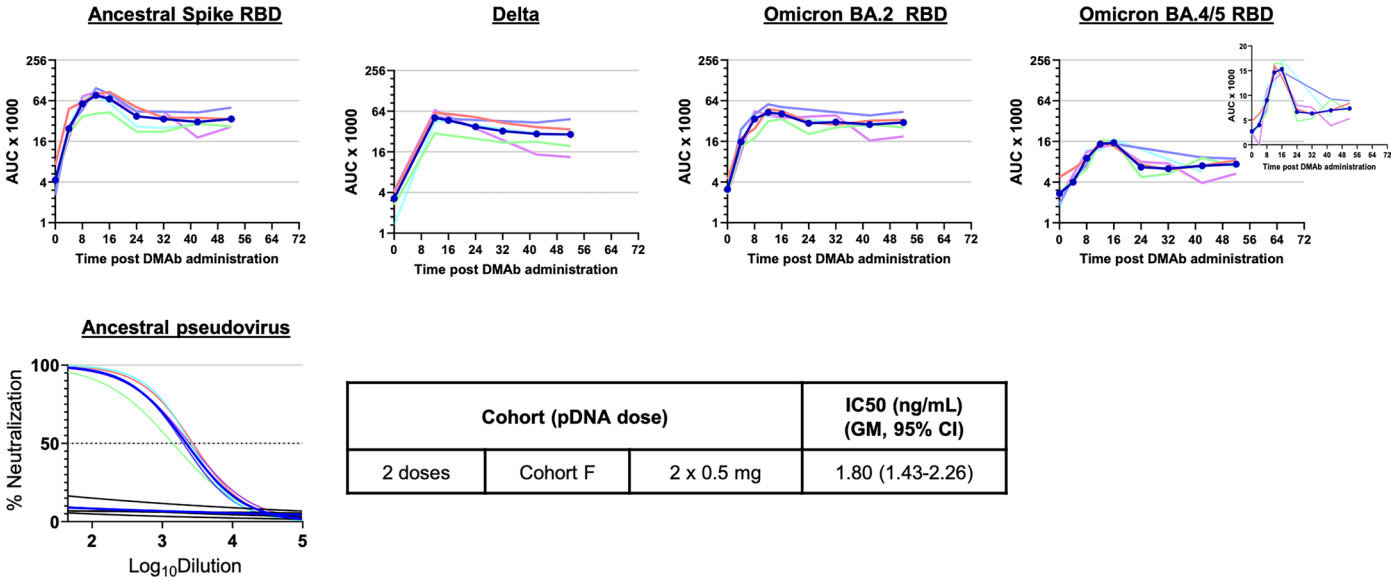
Extended Data Fig. 4 | Longitudinal serum concentration of *in vivo*-expressed DMAb delivered with a modified EP pulse pattern (OpBlock 0070). Serum DMAb concentrations (ng/mL) measured using qualified quantitative binding assays are shown for study participants receiving two doses of 0.5 mg of SARS-CoV-2 DMAb delivered using electroporation with a truncated pulse pattern (OpBlock 0070). Serum concentrations are shown for AZD5396 (left), AZD8076 (middle), and total DMAb (right). Arrows indicate dosing events.

https://doi.org/10.1038/s41591-025-03969-0



Extended Data Fig. 5 | Longitudinal binding by dose of *in vivo*-expressed DMAb against SARS-CoV-2 Delta, Omicron BA.2 and BA.4/5 RBDs. An anti-YTE antibody was used to capture DMAb from participant sera. Binding was detected with biotinylated Spike receptor binding domains (RBD). Graphs are plotted as area under the curve (AUC x 1000) for individual participants and the group mean is shown in navy blue.

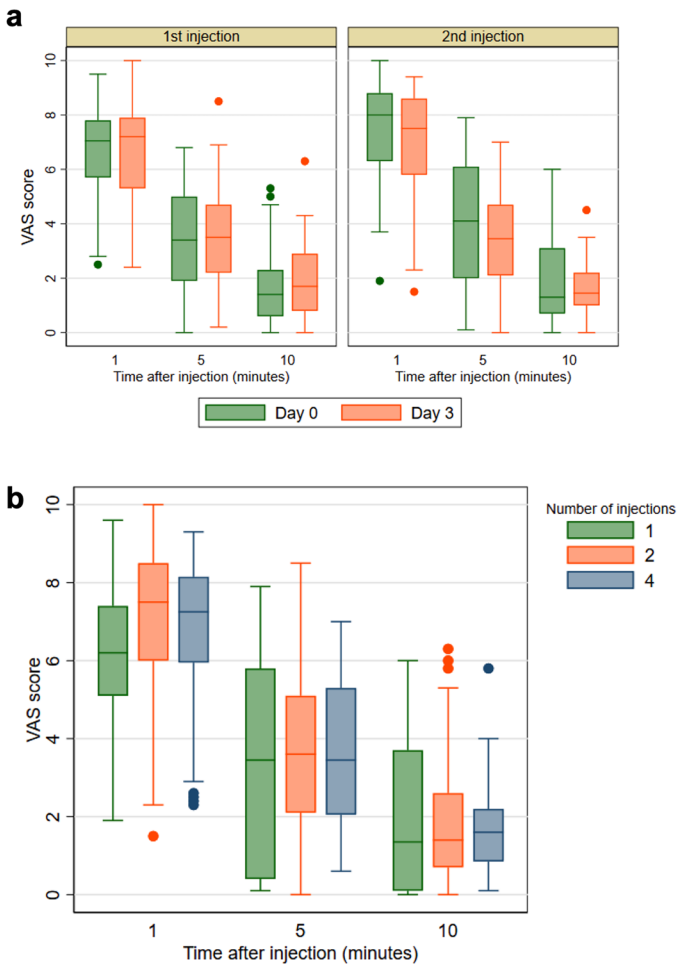
https://doi.org/10.1038/s41591-025-03969-0



Cohort (pDNA dose)			IC50 (ng/mL) (GM, 95% CI)
2 doses	Cohort F	2 x 0.5 mg	1.80 (1.43-2.26)

Extended Data Fig. 6 | *In vivo*-expressed DMAbs delivered with modified EP parameters (OpBlock 0070) bind to SARS-CoV-2 Spike RBDs. An anti-YTE antibody was used to capture DMAb from participant sera. Binding was detected with biotinylated ancestral, Delta, BA.2, or BA.4/BA.5 Spike RBDs. Graphs are plotted as area under the curve (AUC x 1000) for individual participants and the group mean is shown in navy blue.

https://doi.org/10.1038/s41591-025-03969-0



Extended Data Fig. 7 | Visual Analog Scale Scores. A visual analog scale (0 to 10 cm) was used to evaluate pain following the electroporation procedure. Pain typically resolved within minutes of administration (panel **a**). No significant differences in pain intensity were observed with repeated administrations (panel **b**). Boxes represent the interquartile range (IQR) with the horizontal line indicating the median; whiskers show the upper and lower adjacent values (1.5 times the IQR above/below the upper/lower quartile), and individual outlier values are shown as dots.

https://doi.org/10.1038/s41591-025-03969-0

Extended Data Table 1 | Overview of study cohorts (pDNA dose/regimen)

OpBlock 0078 Cohorts

Cohort #	Protocol Cohort	pDNA injected/ dose (at separate sites)		n	Dose schedule (Day)	Total dose of each pDNA (mg)	Hylenex dose*
		pAZD5396	pAZD8076				
1 dose							
1	A1	0.5 mg	0.5 mg	4#	0	0.5 mg	68 U
2	A2	1 mg	1 mg	3	0	1 mg	135 U
2 doses							
3	D	0.25 mg	0.25 mg	5	0, 3	0.5 mg	34 U
4	B	0.5 mg	0.5 mg	6	0, 3	1 mg	68 U
5	C	1 mg	1 mg	6	0, 3	2 mg	135 U
6	E	2 mg	2 mg	5	0, 3	4 mg	75 U
4 doses (2x2)							
7	G	0.5 mg	0.5 mg	5	0, 3, 28, 31	2 mg	75 U

OpBlock 0070 Cohort

8	F	0.5 mg	0.5 mg	5	0, 3	1 mg	75 U

- Hyaluronidase was dosed as ratio of 1:9 in volume for cohorts A-D and as a fixed dose of 75U for cohorts E-G

One participant in cohort B received only on dose o the product, but was otherwise followed per protocol and was analyzed as part of cohort A1

https://doi.org/10.1038/s41591-025-03969-0

Extended Data Table 2 | T_{max} and C_{max} values for individual DMAbs

	Total DMAb		AZD5396		AZD8076	
Participant ID	Tmax (weeks)	Cmax (ng/mL)	Tmax (weeks)	Cmax (ng/mL)	Tmax (weeks)	Cmax (ng/mL)
Cohort A1: 1 x 0.5 mg						
02	24	378.9	24	231.6	24	147.4
03	12	338.0	62	125.3	12	237.5
04	8	294.1	42	96.4	8	250.9
14	8	14.3	NA	0.0	16	14.3
Cohort Mean:	**13.0**	**256.3**	**42.7**	**113.3**	**15.0**	**162.5**
Cohort A2: 1 x 1 mg						
06	16	124.1	12	50.9	16	76.0
07	52	148.7	52	126.0	52	22.7
10	8	202.6	72	67.8	8	155.8
Cohort Mean:	**25.3**	**158.5**	**45.3**	**81.6**	**25.3**	**84.8**
Cohort D: 2 x 0.25 mg						
32	62	297.3	62	162.6	72	135.3
34	62	161.4	62	80.5	62	80.9
35	8	331.5	62	159.5	8	194.4
36	8	204.5	8	125.0	6	82.5
37	12	148.6	42	43.0	6	117.7
Cohort Mean:	**30.4**	**228.7**	**47.2**	**114.1**	**30.8**	**122.2**
Cohort B: 2 x 0.5 mg						
11	52	315.1	52	291.7	52	23.3
12	52	489.1	72	149.6	12	359.9
13	32	615.1	32	267.0	16	394.8
18	8	185.5	8	112.8	42	96.8
19	6	622.0	6	192.6	6	429.4
20	8	252.4	8	74.2	5	178.5
Cohort Mean:	**26.3**	**413.2**	**29.7**	**181.3**	**22.2**	**247.1**
Cohort C: 2 x 1 mg						
23	32	484.9	32	307.5	32	177.4
24	8	345.0	8	207.8	12	139.5
25	42	527.7	52	322.9	12	265.9
21	52	428.3	52	293.2	32	139.4
27	72	660.9	72	359.3	12	332.8
28	8	284.1	8	121.7	8	162.4
Cohort Mean:	**35.7**	**455.2**	**37.3**	**268.7**	**18.0**	**202.9**
Cohort E: 2 x 2 mg						
38	8	242.8	8	98.8	8	144.0
39	6	110.4	6	74.2	6	36.1
40	16	696.9	42	312.7	12	475.4
41	72	501.6	72	337.6	12	187.3
44	72	293.4	12	211.5	8	110.4
Cohort Mean:	**34.8**	**369.0**	**28.0**	**207.0**	**9.2**	**190.6**
Cohort G: 4 x 0.5 mg						
55	12	646.4	12	393.8	8	287.2
57	12	1109.4	12	590.7	12	518.7
58	72	1093.8	72	920.0	12	286.9
59	72	1610.8	72	605.3	72	1004.8
61	8	690.2	8	288.1	8	402.1
Cohort Mean:	**35.2**	**1030.1**	**35.2**	**559.6**	**22.4**	**499.9**

https://doi.org/10.1038/s41591-025-03969-0

Extended Data Table 3 | SARS-CoV-2 RBD background in participants on study entry

Cohort	Anti-Spike RBD IgG Endpoint GMT (95% CI)			Pseudoneutralization assay		
	Anc.	BA.4/BA.5	JN.1	Ancestral	BA2	BA4/5
1 dose						
Cohort A1: 1 x 0.5 mg	25600 (4227-155043)	4525 (548-37402)	1527 (609-3830)	7987 (3882-16430)	1045 (118-9219)	902 (118-6915)
Cohort A2: 1 x 1 mg	40637 (5565-296764)	8686 (218-346437)	68.4 (0-631696)	20594 (2237-189601)	9673 (873-107180)	3765 (904-15680)
2 doses (total DNA)						
Cohort D: 2 x 0.25mg (0.5 mg)	33779 (15644-72941)	8445 (1281-55654)	1326 (201-8706)	3064 (1691-552)	3310 (1262-8681)	2421 (1066-5500)
Cohort B: 2 x 0.5 mg (1 mg)	29824 (3672-242223)	3200 (352-29064)	1174 (394-3499)	6261 (1357-28882)	3078 (314-30125)	1179 (83-16754)
Cohort C: 2 x 1 mg (2 mg)	20319 (4861-84938)	5080 (927-27827)	54 (2-1396)	8970 (4199-19162)	11990 (3721-38634)	5899 (2228-15619)
Cohort E: 2 x 2 mg (4 mg)	19401 (4596-81899)	7687 (2055-28754)	1456 (419-5057)	7620 (1961-29614)	3405 (489-23719)	3509 (397-31036)
4 doses (total DNA)						
Cohort G: 4 x 0.5 mg (4 mg)	44572 (6262-317256)	10143 (1514-55654)	3046 (462-20055)	5260 (3113-8887)	7806 (849-71764)	11405 (1024-126991)
2 doses - Alternative delivery parameters (total DNA)						
Cohort F: 2 x 0.5 mg (1 mg)	40573 (9405-175035)	11143 (2390-51956)	4415 (776-25106)	16517 (2050-133106)	31468 (4095-241792)	8336 (1617-42979)

GMT = geometric mean titer, CI = confidence interval

nature portfolio

Corresponding author(s): Pablo Tebas
David Weiner

Last updated by author(s): 8/15/2025

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

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☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *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	Not applicable
Data analysis	All statistical analyses were performed using STATA (version 16).

For manuscripts utilizing custom algorithms or software that are central to the research but 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 Portfolio guidelines for submitting code & software for further information.

Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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All requests for raw and analyzed clinical trial data and materials will be promptly reviewed by the corresponding authors (P.T. and D.B.W.) to determine if they are subject to intellectual property or confidentiality obligations, particularly with respect to planned pharmacokinetic modeling analyses to be reported in a subsequent manuscript. Any data and materials that can be shared will be released via a material transfer agreement (requested to David B. Weiner and the Wistar

Institute, for review by the Wistar Legal group). Sequences for the plasmids pAZD5396 and pAZD8076 have been submitted under patent no. WO2023064841A1. Plasmids can be shared upon completion of a material transfer agreement with the corresponding authors.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	The sex of the participants was reported in the paper.
Reporting on race, ethnicity, or other socially relevant groupings	The race of the participants was reported based on self-described characterization, following U.S. Census categories.
Population characteristics	The age of the participants was reported in the study.
Recruitment	61 healthy adults were screened; of these, 44 participants received at least one dose of the investigational DNA plasmids pAZD5396 and/or pAZD8076. Sixteen individuals did not meet eligibility criteria due to timing constraints (n = 5), BMI ineligibility (n = 3), active COVID-19 infection (n = 2), other medical exclusions (n = 4), or personal/external reasons (n = 2). One participant withdrew consent before completing screening
Ethics oversight	Institutional Review Board of the University of Pennsylvania

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

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

Sample size	This Phase 1 study was not powered for hypothesis testing. Cohort sizes were determined based on pre-specified dose-limiting toxicity (DLT) monitoring parameters, consistent with FDA guidance for first-in-human clinical trials. Participants were enrolled in cohorts of n=3, 5, or 6 depending on dose level, with a target DLT threshold of 30% used to guide cohort expansion or modification.". No formal power analysis was performed, as the study focused on estimation rather than hypothesis testing.
Data exclusions	All dosed participants were included in the Safety analysis. Participants that received a single dose of the product were replaced as per protocol.
Replication	See detailed methods section
Randomization	Not applicable
Blinding	Not applicable

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☐ Eukaryotic cell lines
☐	☐ Palaeontology and archaeology
☐	☐ Animals and other organisms
☐	☒ Clinical data
☐	☐ Dual use research of concern
☐	☐ Plants

Methods

n/a	Involved in the study
☐	☐ ChIP-seq
☐	☐ Flow cytometry
☐	☐ MRI-based neuroimaging

Antibodies

Antibodies used

• HRP-conjugated goat anti-human IgG (polyclonal) – Southern Biotech, Catalog #2049-05, used at 1:8,000 dilution.
• HRP-conjugated streptavidin – Thermo Scientific, Catalog #N100, used at 1:10,000 dilu-tion.
AstraZeneca research-grade antibodies
• Anti-YTE monoclonal antibody, Clone 23F7.1 – AstraZeneca, used at 1–2 µg/mL.
• Anti-idiotype monoclonal antibody, Clone M54-2E9 – AstraZeneca, used at 1 µg/mL.
• Anti-idiotype monoclonal antibody, Clone M16H5.1 – AstraZeneca, used at 1 µg/mL.

Validation

The following antibodies were used in binding ELISAs, pseudovirus neutralization assays, and PK/ADA assays:
Commercial antibodies
• HRP-conjugated goat anti-human IgG (polyclonal) – Southern Biotech, Catalog #2049-05, used at 1:8,000 dilution.
• HRP-conjugated streptavidin – Thermo Scientific, Catalog #N100, used at 1:10,000 dilu-tion.
AstraZeneca research-grade antibodies
• Anti-YTE monoclonal antibody, Clone 23F7.1 – AstraZeneca, used at 1–2 µg/mL.
• Anti-idiotype monoclonal antibody, Clone M54-2E9 – AstraZeneca, used at 1 µg/mL.
• Anti-idiotype monoclonal antibody, Clone M16H5.1 – AstraZeneca, used at 1 µg/mL.
Anti-YTE mAb (Clone 23F7.1), anti-idiotype clone M54-2E9, and anti-idiotype clone M16H5.1 were provided by AstraZeneca and have been previously described 13. All primary reagents were confirmed by ELISA. The anti-YTE mAb demonstrated specific binding to YTE-containing AZD5396 and AZD8076 but not to control human IgG serum. Anti-idiotype clone M54-2E9 binds specifically to AZD5396, and clone M16H5.1 binds specifically to AZD8076. For all ELISAs, recombinantly purified anti-AZD5396 and anti-AZD8076 antibodies were used as standards to confirm reagent specificity.
All antibodies were validated either by the manufacturer, in prior peer-reviewed publications, or in-house during assay development.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.

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Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

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Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

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Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

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Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

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Indicate where the specimens have been deposited to permit free access by other researchers.

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If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

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Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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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	ClinicalTrials.gov identifier: NCT05293249
Study protocol	Uploaded
Data collection	Between May 2022 and Febrary 2024, 61 healthy adults were screened; of these, 44 participants received at least one dose of the investigational DNA plasmids pAZD5396 and/or pAZD8076.
Outcomes	The primary endpoints of the study were safety and pharmacokinetics; exploratory analyses included antigen binding and virus neutralization, and post hoc analysis assessed anti-drug antibodies

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Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session
(e.g. UCSC)

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

☐ 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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.