Early evaluation of the safety, reactogenicity, and immune response after a single dose of modified vaccinia Ankara–Bavaria Nordic vaccine against mpox in children: a national outbreak response

Shamez N Ladhani*, Alexander C Dowell*, Scott Jones, Bethany Hicks, Cathy Rowe, Jusnara Begum, Dagmar Wailblinger, John Wright, Stephen Owens, Ailsa Pickering, Benjamin Shilltoe, Paddy McMaster, Elizabeth Whittaker, Jianmin Zuo, Annabel Powell, Gayatri Amirthalingam, Sema Mandal, Jamie Lopez-Bernal, Mary E Ramsay, Neave Kissane, Michael Bell, Heather Watson, David Ho, Bassam Hallis, Ashley Otter†, Paul Moss†, Jonathan Cohen†

Summary

Background In response to a national mpox (formerly known as monkeypox) outbreak in England, children exposed to a confirmed mpox case were offered modified vaccinia Ankara–Bavaria Nordic (MVA-BN), a third-generation smallpox vaccine, for post-exposure prophylaxis. We aimed to assess the safety and reactogenicity and humoral and cellular immune response, following the first reported use of MVA–BN in children.

Methods This is an assessment of children receiving MVA–BN for post-exposure prophylaxis in response to a national mpox outbreak in England. All children receiving MVA–BN were asked to complete a post-vaccination questionnaire online and provide a blood sample 1 month and 3 months after vaccination. Outcome measures for the questionnaire included reactogenicity and adverse events after vaccination. Blood samples were tested for humoural, cellular, and cytokine responses and compared with unvaccinated paediatric controls who had never been exposed to mpox.

Findings Between June 1 and Nov 30, 2022, 87 children had one MVA–BN dose and none developed any serious adverse events or developed mpox disease after vaccination. Post-vaccination reactogenicity questionnaires were completed by 45 (52%) of 87 children. Their median age was 5 years (IQR 5–9), 25 (56%) of 45 were male, and 22 (49%) of 45 were White. 16 (36%) reported no symptoms, 18 (40%) reported local reaction only, and 11 (24%) reported systemic symptoms with or without local reactions. Seven (8%) of 87 children provided a first blood sample a median of 6 weeks (IQR 6·0–6·5) after vaccination and five (6%) provided a second blood sample at a median of 15 weeks (14–15). All children had poxvirus IgG antibodies with titres well above the assay cutoff of OD_{450nm} 0·1926 with mean absorbances of 1·380 at six weeks and 0·9826 at 15 weeks post-vaccination. Assessment of reactivity to 27 recombinant vaccina virus and monkeypox virus proteins showed humoral antigen recognition, primarily to monkeypox virus antigens B6, B2, and vaccina virus antigen B5, with waning of humoral responses observed between the two timepoints. All children had a robust T-cell response to whole modified vaccinia Ankara virus and a select pool of conserved pan-Poxviridae peptides. A balanced $CD4^+$ and $CD8^+$ T-cell response was evident at 6 weeks, which was retained at 15 weeks after vaccination.

Interpretation A single dose of MVA–BN for post-exposure prophylaxis was well-tolerated in children and induced robust antibody and cellular immune responses up to 15 weeks after vaccination. Larger studies are needed to fully assess the safety, immunogenicity, and effectiveness of MVA–BN in children. Our findings, however, support its ongoing use to prevent mpox in children as part of an emergency public health response.

Funding UK Health Security Agency.

Introduction

Mpox (formerly known as monkeypox) disease is a zoonotic infection that is endemic in central and west Africa. Cases in non-endemic countries are usually associated with recent travel to endemic countries or contact with an infected person or animal from an endemic country.[1] Between 2018 and 2021, there were seven mpox cases in the UK; four were imported, two were cases in household contacts, and one was a health-care worker caring for an imported case.[2] In May, 2022, the UK had three different mpox incidents,[3] all due to clade II (West African), which is associated with less severe disease than clade I (Central African).[1] The first incident was a travel-associated case from Nigeria with no further spread. The second incident was not travel-related and likely to have been acquired through local transmission within the UK, with spread limited to the same household.[4] The third and largest

(P McMaster MRCP);
Department of Paediatric Infectious Diseases, Imperial College Healthcare NHS Trust, London, UK (E Whittaker PhD);
Section of Paediatric Infectious Diseases, Imperial College London, London, UK (E Whittaker); **Paediatric Infectious Diseases Department, Evelina London Childrens' Hospital, London, UK** (N Kissane MBChB, M Bell BSc, H Watson BSc, D Ho MRCPCH, J Cohen PhD)

Correspondence to:
Dr Shamez Ladhani,
Immunisation Department, UK Health Security Agency,
London NW9 5EQ, UK
shamez.ladhani@ukhsa.gov.uk

Research in context

Evidence before this study
We searched PubMed and preprint servers SSRN and Medvrix with the terms ("monkeypox" or "mpox") AND ("child" or "children") in the title or abstract for publications published from database inception to Dec 31, 2022. Compared with adults, little is known about risks, presentations, and outcomes of mpox in children, especially in non-endemic countries. Some data suggest that young children, pregnant women, and immunocompromised people might be at increased risk of severe mpox disease. There are no studies on modified vaccinia Ankara–Bavaria Nordic (MVA-BN) for protection against smallpox or mpox in children. In adults, a single dose of MVA-BN has been shown to be highly protective against mpox disease among gay, bisexual, and other men who have sex with men in the current global mpox outbreak.

Added value of this study
This is the first study to evaluate the use of MVA-BN in children. Although only a small proportion agreed to have post-vaccination blood samples, the findings of robust and sustained humoral and cellular responses in all vaccinated children is reassuring and supports the use of MVA-BN in children during the current global mpox outbreak. Our results need to be replicated in a larger cohort of unexposed children.

Implications of all the available evidence
One dose of MVA-BNA is immunogenic and induces robust humoral and cellular responses against mpox in children. If our findings are confirmed in larger studies, MVA-BN could play a vital role in preventing childhood mpox in endemic and non-endemic countries.

incident involved mainly gay, bisexual, and other men who have sex with men (GBMSM) and spread rapidly across many countries, leading WHO to declare a public health emergency of international concern on July 23, 2022.[5] This was the first time that chains of mpox transmission were reported in non-endemic countries. Cases in the UK increased rapidly until mid-July, 2022, before plateauing and then declining.[6] As of Nov 28, 2022, there were 3547 mpox cases confirmed in England, including 52 women and one child (a neonate with mpox and adenovirus coinfection).[2]

Mpox typically causes a mild self-limiting illness, with spontaneous and complete recovery within 3 weeks.[1] On the basis of a small amount of published literature, young children appear to be at increased risk of severe disease, along with pregnant women and immunocompromised people.[7] Because mpox is considered a high consequence infectious disease in the UK,[8] confirmed cases were initially hospitalised for isolation, observation, and treatment, with extensive efforts made to identify and isolate close contacts to contain the outbreak. Close contacts were also offered vaccination with modified vaccinia Ankara–Bavarian Nordic (MVA–BN) for post-exposure prophylaxis.[9]

MVA–BN is a third-generation vaccine containing a highly-attenuated Chorioallantois vaccinia virus Ankara poxvirus strain that is unable to replicate in humans and cannot cause disease.[9] MVA–BN was licensed by the European Medicines Agency in 2013 for smallpox prevention. Previous studies have shown that two doses are immunogenic, generating antibody titres considered protective against smallpox and, by extrapolation, against mpox. MVA–BN also prevented lethal mpox in primate challenge models, with protection beginning from 6 days after one dose.[10]

Unlike the live smallpox vaccine, there are little data on whether MVA–BN can prevent or modify disease when given post-exposure. Antibody titres at 14 days after one

MVA vaccine dose were equivalent to a live smallpox vaccine (ACAM2000), with similar seroconversion rates of 90·8% and 91·8%, respectively.[11] On the basis of this immunological response, contacts of a confirmed case were offered rapid vaccination to prevent infection or to modify disease severity.[12]

MVA–BN, however, has not been administered to children of any age in pre-licensure or post-licensure studies and is not licensed in children. Other paediatric vaccines have used MVA as a vector, often at a higher dose than MVA–BN, with a reassuring safety and reactogenicity profile.[13–15] Given their increased risk of severe disease, MVA–BN was also recommended for post-exposure prophylaxis in children in the UK exposed to a confirmed mpox case.[12] We aimed to assess the safety, reactogenicity, immunogenicity, and cellular immune responses following a single MVA–BN dose in children vaccinated as post-exposure prophylaxis during the 2022 clade IIb mpox outbreak in England. Here, we report our preliminary findings.

Methods
Study design and participants
We evaluated vaccine uptake, reactogenicity, and immune responses in children offered MVA–BN for post-exposure prophylaxis as part of a national mpox outbreak in England. The UK Health Security Agency initiated post-vaccine implementation surveillance in children receiving MVA–BN in July 2022. Children aged 0–16 years exposed to a laboratory-confirmed mpox case in a household or educational or other setting were offered a single MVA–BN dose as post-exposure prophylaxis, ideally within 4 days of first exposure but up to 14 days after last exposure, as part of clinical and public health management of close contacts.[12] MVA–BN (0·5 mL containing $0·50–3·95 \times 10^8$ infectious units of MVA–BN live virus) was administered by subcutaneous or intramuscular injection into the deltoid area of the upper

	Cohort (n=45)
Sex	
Male	25 (56%)
Female	20 (44%)
Ethnicity	
White	22 (49%)
Black	5 (11%)
Asian	5 (11%)
Mixed	5 (11%)
Other	5 (11%)
Missing	3 (7%)
Underlying health conditions	
Asthma	1 (2%)
Eczema	3 (7%)
Hay fever	7 (16%)
Other allergies	5 (11%)
Other medical conditions	3 (7%)
Outcome of vaccination	
Contacted doctor for advice	3 (7%)
Hospitalised	0

Data are n (%) or n/N (%).

Table 1: Demographics of children receiving modified vaccinia Ankara–Bavaria Nordic

arm or, for small children, anterolateral thigh.[3] The children were vaccinated through national vaccine hubs and followed up by local paediatric infectious diseases and health protection teams for 28 days for serious adverse events and development of mpox disease. Additionally, parents were asked to complete a questionnaire on solicited reactogenicity and adverse events during the first seven days after vaccination. Children receiving MVA–BN were invited to give a blood sample (up to 10 mL) around 1 month and 3 months after vaccination for serology and cellular immunity tests. Because this study recruited participants following an emergency public health intervention, the timing of blood sampling was flexible. Control samples for serology were obtained from unvaccinated adults before smallpox vaccination or from unvaccinated children (median age 3 years, IQR 1–5 years) as described previously.[16] Control samples for cellular immunity were obtained from unvaccinated children enrolled in the Born in Bradford study, an ongoing longitudinal study of immune responses.[17] Samples were couriered to designated laboratories on the same day for processing. Informed written consent was taken from parents before sampling. The protocol (appendix p 8) was approved by Public Health England Research Ethics and Governance Group (reference NR0333; July 28, 2022).

Procedures

Serology samples were collected in BD Serum Separating Tubes and, after serum separation, run on a 96-well plate coated with a pool of recombinant vaccinia virus and monkeypox virus proteins as previously described.[16] Individual recombinant antigens used were: vaccinia virus A27 (V.A27), A33 (V.A33), and B5 (V.B5), and monkeypox virus proteins A5, A14, A26, A27, A29, A33, A35, A36, A44, B2, B5, B6, C15, C18, C19, D13, D14, E8, F3, H3, I1, L1, L4, and M1. Vaccinia virus proteins B5, A27, and A33 are homologues of the monkeypox virus proteins B6, A29, and A35, respectively. An OD_{450nm} of 0·1926 was used as a positive cutoff on the basis of previously calculated receiver operating characteristic analysis for this assay.[16] Antigen pool-coated plates were also used to determine endpoint titres (dilution required for sample to achieve an absorbance of 0·1926) of each sample. This was calculated by fitting a sigmoid 4-parameter (4PL) model to the absorbances of sample serially diluted one in four and using the cutoff of OD_{450nm} 0·1926.

For peripheral blood and mononuclear cell (PBMC) preparation, lithium heparin blood tubes were processed within 24 h of collection. Briefly, tubes were spun at 300 g for 10 min before removal of plasma. Remaining blood was diluted with Roswell Park Memorial Institute (RPMI) medium and PBMC isolated on a Sepmate Ficoll density gradient (Stemcell, Cambridge, UK), cells were washed with RPMI, and subsequent cultures used RPMI + 10% batch tested fetal bovine serum.

T-cell responses were measured using an IFN-γ ELISpot Pro kit (Mabtech, Stockholm, Sweden). A PepMix pool (JPT, Berlin, Germany) containing 127 peptides containing defined HLA class I-restricted and class II-restricted T-cell epitopes peptides from selected proteins of Poxviridae. Of these, 113 peptides are shared between monkeypox virus strain Zaire-96-I-16 and MVA, while 107 peptides are shared between the COP-58 West African monkeypox virus strain and MVA. Briefly, fresh PBMC were rested for 4 h before assay to acclimatise freshly isolated cells to media, $0·3 \times 10^6$ PBMCs were added in duplicate to wells containing either PepMix at a final individual peptide concentration of 1 µg/mL, DMSO (negative) control, or a single well containing anti-CD3 (positive). Samples were incubated for 16–18 h. Plates were developed following the manufacturer's instructions and read using an AID plate reader (AID, Strassberg, Germany). Results are presented as spot-forming cells per million PBMC. Results are normalised by subtraction of the value of the negative-control wells from the test wells.

Purified MVA virus was obtained from Graham Taylor (University of Birmingham). MVA contained an irrelevant transgene, ovalbumin (NC_052533.1), previously used as a negative control. MVA was used to infect PBMCs as previously described.[18] Briefly, PBMCs were either infected with MVA at a multiplicity of infection of 3 or mock infected for 2 h. Cells were then washed with culture media before assay. Two approaches were used: PBMCs were added in duplicate to wells in an IFNγ

ELISpot plate (Mabtech, Stockholm, Sweden), and PBMCs were cultured and assessed by flow cytometry in an Activation Induced Marker (AIM) assay.

For activation induced marker assays, $1 \cdot 0$–$1 \cdot 5 \times 10^6$ MVA (6×10^6 MVA per mL) or mock infected PBMC were plated into a 96-well round bottom plate and cultured for 16–18 h. 1 µg/mL purified anti-CD40 antibody (BioLegend, Cambridge, UK) was included in all cultures. Following stimulation, supernatant was removed and stored at –80°C for future cytokine analysis. Cells were transferred to 5 mL polypropylene tubes and washed with cold wash buffer (PBS+0·5%, BSA+2mM EDTA), Tru-Fx Fc-block was added for 5 min (Biolegend, Cambridge, UK) before addition of an antibody mastermix including 50 µL of brilliant staining buffer (BD Biosciences, Eysins, Switzerland) per sample (appendix p 6). The cell suspension was stained at 4°C for 30 min. Cells were then washed and fixed with 1·6% paraformaldehyde for 30 min at room temperature in the dark, washed, and run on a BD Symphony A3 flow cytometer (BD Biosciences). Data were collected using BD FACS Diva 8 and analysis performed in FlowJo version 10.7.1.

Cytokine profiles were analysed in MVA-infected and mock-infected AIM culture supernatant using a Legendplex Human CD8/NK (13-plex) panel kit (BioLegend). Samples were run on a BD LSR II flow cytometer (BD Biosciences) and acquired using BD FACS Diva 7. A standard curve was produced and unknown sample concentrations determined using LEGENDplex software version 8 (BioLegend).

Statistical analysis

Participant demographics and reactogenicity data are mainly descriptive and presented as numbers and proportions. Continuous variables that did not follow a normal distribution are described as medians with IQRs. Paired two-tail t tests were used to determine statistical differences in endpoint antibody titres in children who provided blood samples at both timepoints. A Kruskal-Wallis test with Dunn's multiple comparisons test was used to compare ELISpot data from vaccinated and unvaccinated children. The association between antibody and cellular data used a linear regression. Statistical analyses were conducted using GraphPad Prism version 9.2.0.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between June 1 and Nov 30, 2022, 87 children had one MVA–BN dose for post-exposure prophylaxis against mpox and none developed any serious adverse events or mpox disease post-vaccination. Of the 87 children,

	Mild	Moderate	Severe
Local (at the injection site)			
Pain	18 (40%)	0	0
Swelling	18 (40%)	0	0
Itch	5 (11%)	0	0
Systemic			
Feeling hot	1 (2%)	1 (2%)	1 (2%)
Fever	0	1 (2%)	1 (2%)
Tiredness	3 (7%)	2 (4%)	0
Headache	2 (4%)	0	0
Rash	2 (4%)	2 (4%)	0
Nausea	0	0	1 (2%)
Appetite loss	0	0	1 (2%)
Muscle aches	1 (2%)	0	0
Itching all over body	1 (2%)	0	0
Abdominal pain	1 (2%)	0	0

Table 2: Reactogenicity in children up to 7 days after one dose of modified vaccinia Ankara–Bavaria Nordic vaccine (n=45)
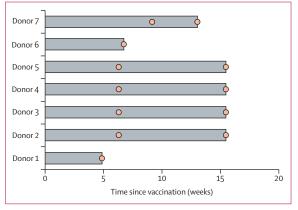

Figure 1: Blood sampling timepoints for seven children vaccinated with modified vaccinia Ankara–Bavaria Nordic for post-exposure prophylaxis during the 2022 mpox outbreak in England

45 completed the 7-day follow-up questionnaire. Their median age was 5 years (IQR 5–9 years), 25 (56%) of 45 were male, and 22 (49%) of 45 were White (table 1).

No symptoms were reported by 16 (36%) of 45 children, whereas 18 (40%) reported local reaction only (mainly swelling [20 (44%)] and pain [18 (40%)] at injection site) and 11 (24%) reported systemic symptoms (mainly rash [five (11%)] and fever or feeling hot [five (11%)]) with or without local reactions (table 2). Four of the 11 children who experienced systemic symptoms were reported as moderate-to-severe compared with none of 25 reporting local symptoms. Three parents contacted the vaccination team for advice on management of post-vaccination reactions, but none required a medical visit or hospitalisation.

Seven children, including four siblings, provided at least one blood sample. Four were household contacts of a confirmed case and three were contacts of a confirmed case in an educational setting. The median time to first
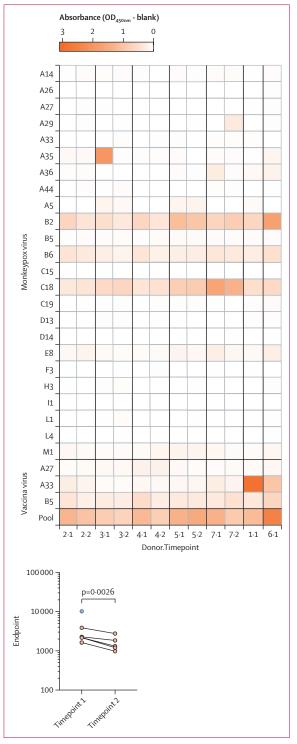
Figure 2: **Antibody responses 6 weeks and 15 weeks after a single dose of MVA-BN in children**

(A) Heatmap of samples to panels of monkeypox virus and vaccina virus recombinant antigens, with absorbance values shown for serum samples from individuals at two different timepoints. (B) Samples serially diluted on the antigen pool and interpolated endpoint titres (dilution required to reach cutoff OD) with paired samples connected. MVA-BN=modified vaccinia Ankara-Bavaria Nordic.

sampling after vaccination was 6 weeks (44 days, IQR 44·0–45·5) and to second sampling was 15 weeks (108 days, IQR 108–108; figure 1). When assessing antibody responses, the absorbances of each sample were well above the assay cutoff of OD_{450nm} 0·1926, demonstrating the presence of poxvirus IgG. When assessing reactivity to the 27 recombinant vaccinia virus and monkeypox virus proteins, samples primarily showed reactivity to monkeypox virus antigens B6, B2, C18, and vaccinia virus antigen B5 (figure 2A; appendix p 2). Sample reactivity to vaccinia virus A33 and A35 was variable. Using the pooled antigen ELISA, all samples showed reactivity well above the assay cutoff of OD_{450nm} 0·1926 with mean absorbances of 1·380 at 6 weeks (SD 0·5140) and 0·9826 at 15 weeks (0·3206) after vaccination (figure 2A). Serial dilution of samples on plates coated with the antigen pool (appendix p 3) showed a significant decrease in endpoint titres 15 weeks (1624, SD 710·9) compared with 6 weeks (3422, SD 3079) after vaccination (p=0·0026; figure 2B).

Cellular responses against whole MVA virus or a select pool of pan-Poxviridae peptides were first assessed by IFNγ ELISpot. After one MVA–BN dose, all seven children had a cellular response by IFNγ ELISpot, but the magnitude of response varied between donors. Response to whole MVA was a median of 4·5 times higher (IQR 2·4–16·7) than the select pool of pan-Poxviridae peptides, likely due to the select nature of the peptide pool. The response to both stimulations, however, were well-correlated, showing cross-reactive responses between vaccine and other pox virus including monkeypox virus (figure 3A). The median response to MVA virus was 360 spot-forming cells (SFC) per 10^6 PBMCs (IQR 295–822) at 6 weeks (n=7), reducing to 170 SFC per 10^6 PBMCs (IQR 124–475) by 15 weeks (n=5), compared with 14 SFC per 10^6 PBMCs (IQR 0–54) in unexposed and unvaccinated children (n=9). The median response to pan-Poxviridae peptides was 83 SFC per 10^6 PBMCs (IQR 28–182) at 6 weeks and 70 SFC per 10^6 PBMCs (IQR 26–293) at 15 weeks compared with 0 SFC per 10^6 PBMCs (IQR 0–12) in unexposed and unvaccinated children.

The AIM assay was used to define the phenotype of responding T cells (appendix p 4) and showed a balanced CD4+ and CD8+ T-cell response at 6 weeks, which was retained at 15 weeks after vaccination (figure 3B). The CD4+ T-cell response differed from the CD8+ response at 6 weeks after vaccination. MVA-specific CD4+ T cells were of mixed memory and effector phenotype. This included T memory stem cell (CD45ra+CCR7+CD95+), central memory (CD45ra–CCR7+), and early effector memory (CD45ra–CCR7–CD28+CD27+/–) T cell populations. By contrast, the MVA-specific CD8+ T-cell population was dominated by a late effector memory phenotype (CD45+CCR7–; figure 3C). These distinct phenotypes were maintained at 15 weeks (figure 3D). A comparison of cellular and serology results against B2R

and the pooled antigen found no consistent correlation of cellular and serology responses (appendix pp 5, 7).

Cytokine profiling of AIM assay culture supernatant to determine the functional MVA-response of cells at 6 weeks after vaccination showed a predominately Th1 cytokine profile (median IFNγ 2869 pg/mL [IQR 544–10 422], IL-4 3 pg/mL [2–4], and IL-17A 7 pg/mL [6–12]; figure 3E). There was also robust release of granzyme (median 1639 pg/mL, IQR 486–3523) and perforin (254 pg/mL, 87–632), demonstrating cytotoxic potential of responding cells. This profile was maintained 15 weeks after vaccination, indicating a durable effector response.
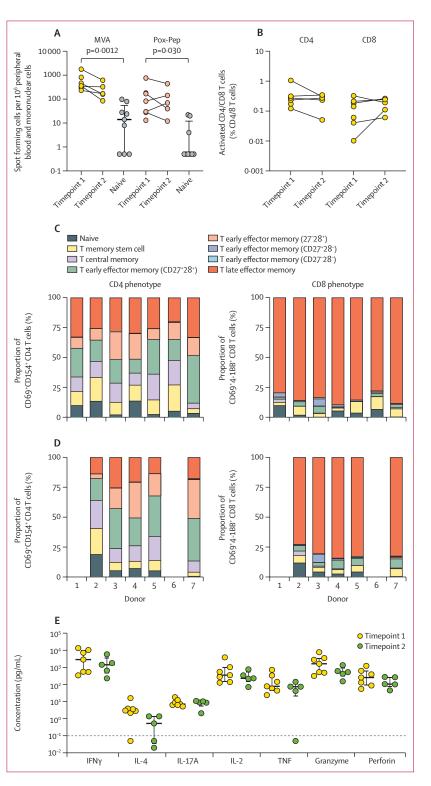
Discussion

We report the first ever use of MVA–BN as post-exposure prophylaxis against mpox disease in children during a national outbreak in England. Although our study involved a small sample size and opportunistic follow-up of children vaccinated as part of a national outbreak response, our findings are reassuring in terms of the reactogenicity, safety, and immunogenicity of a vaccine that had previously never been used in children. None of the 87 children had a serious adverse event or developed mpox disease after vaccination, and most children described no symptoms or mild self-limiting local symptoms of short duration. Seven children provided a blood sample at 6 weeks after vaccination; all were positive for poxvirus IgG and showed specific reactivity to monkeypox virus B2 (EEV type I membrane glycoprotein haemagglutinin), B6 (palmitated 42-kDa EEV glycoprotein), C18 (actin-tail formation), and vaccinia virus B5 (same as monkeypox virus B6). The vaccine also induced robust T-cell responses characterised by a predominantly effector CD8+ T cell population and memory phenotype CD4+ T cells. These responses were well maintained at 15 weeks after vaccination.

Because of a scarcity of data on MVA–BN use in children, we undertook a pragmatic post-implementation evaluation of vaccine responses in children receiving MVA–BN in response to a national outbreak. The UK recommendation to vaccinate children with MVA–BN as part of the national outbreak response was based on safety, reactogenicity, and immunogenicity studies in adults, alongside previous experience with smallpox vaccines and other paediatric MVA vaccines. Since

***Figure 3:* Cellular response following MVA vaccination**
(A) Frequency of cellular response to MVA infection or a pool of conserved Pox-Pep, by IFNγ ELISpot, lines indicate matched samples, at first (n=7) and second (n=5) timepoints. (B) Frequency of MVA-specific T cells responding to MVA infection, CD4 (CD69+CD40L+), and CD8 (CD69+CD137+) T cells. Lines indicate matched samples at the indicated timepoints. (C) Phenotype of responding CD4 (CD69+CD40L+) and CD8 (CD69+CD137+) T cells at the first timepoint after vaccination. Each bar represents an individual donor. (D) Phenotype of responding CD4 and CD8 T cells at the second timepoint after vaccination. Donor numbers are matched across both timepoints. (E) Cytokine production during AIM assay cultures in response to MVA infections at the first (yellow, n=7) and second (green, n=5) timepoints. Cytokine release is normalised to matched mock infected cultures; individual points represent individual donors. AIM=Activation-Induced Marker. MVA=modified vaccinia Ankara. Pox-Pep=Poxviridae immunogenic peptide epitopes.

children were considered to be at risk of severe mpox disease, the benefits of vaccinating close contacts were assessed to outweigh any potential risks of vaccination. Because MVA–BN was offered as part of an outbreak response rather than a clinical trial, both vaccine uptake and participation in post-vaccination surveillance were voluntary.

We recently reported our experience with childhood mpox exposure in four different educational settings.[19] Parents of children aged 2–11 years were offered one MVA–BN dose for their child for post-exposure prophylaxis, but only 21 (11%) of 186 were vaccinated.[19] Parental reasons for vaccine refusal included the long interval between exposure and offer of vaccination, a low perceived risk because of limited exposure to the index case, the distance required to travel to the nearest paediatric mpox vaccine centre, and concerns about scarcity of vaccine safety or effectiveness data in children.

In addition to safety and reactogenicity, we also evaluated humoral and cellular responses in children who provided post-vaccination blood samples. Seraum samples from vaccinated children primarily reacted to monkeypox virus B2, B6, and vaccinia virus B5 antigens, similar to adults after one MVA–BN dose.[16] Vaccinia virus B5 is a homologue of monkeypox virus B6, so similar antibody responses against these two antigens was expected. Vaccinia virus B5 (or monkeypox virus B6) antibodies are also induced after smallpox vaccination with ACAM2000,[20] with robust immune responses after vaccination.[21,22] Monkeypox virus B2 is a homologue of vaccinia virus A56, a membrane glycoprotein that binds vaccinia virus complement-control protein and vaccinia protein K2.[23] Previous work has also shown robust immune responses against A56 protein after vaccination,[23] consistent with our findings in children. We did not detect any antibodies induced by MVA–BN against monkeypox virus A27 in children, consistent with previous observations that the gene encoding this protein is absent from the MVA strain in MVA–BN and only observed in individuals with ACAM2000 vaccination or mpox infection.[16,22] Given that MVA–BN was offered as post-exposure prophylaxis, this finding is important because it indicates that the immune responses in children were vaccine-related rather than following virus exposure. Antibodies against monkeypox virus C18 (vaccinia virus F12) were observed in all samples; however, we have observed reactivity to this antigen in individuals with no prior pox infection or vaccination,[16] suggesting cross-reactive antibodies with other antigens. Using a pooled ELISA antigen, we observed decreasing endpoint titres by 16 weeks after vaccination (p=0·0026), indicating waning of humoral responses.

We also found that all tested children developed robust cellular responses following vaccination. We used a non-replicating MVA vector, similar to MVA–BN, to assess responses to naturally processed antigen. Additionally, using a cross-reactive immunogenic peptide pool conserved between MVA and mpox allowed assessment of mpox-specific responses, although this response likely underestimates cross-reactive responses because of the limited peptide pool and similarities between the two virus sequences. The strong effector CD8+ T-cell response indicates the ability of MVA to induce a cytotoxic T-cell response aiding the clearance of virus-infected cells. We also demonstrated induction of stem cell-like memory T cells, an important subset of multipotent memory T cells with the ability to self-renew. This finding indicates potential life-long memory responses after a single MVA–BN dose. Despite the predominant effector population in our cohort, adults vaccinated with a previous smallpox vaccine (Dryvax; Wyeth Pharmaceuticals, Marietta, PA, USA) maintained cellular responses for decades after vaccination, although an age-dependant waning of the response was evident.[24]

Until the current outbreak, there were no real-world data on human protection against mpox disease by MVA–BN. The vaccine was licensed using immunogenicity and animal studies only, but mass vaccination programmes for high-risk GBMSM in some countries are providing reassuring results even after one dose. In England, we recently estimated MVA–BN vaccine effectiveness against mpox 14 days or more after one dose to be 78% (95% CI 54–89) using the case-coverage method,[25] with Israel reporting an adjusted vaccine effectiveness of 86% (95% CI 59–95) in a similar cohort of high-risk GBMSM.[26] A USA study also reported mpox incidence in unvaccinated individuals eligible for MVA–BN to be 14 times higher than in those receiving at least one vaccine dose, equivalent to a vaccine effectiveness of around 93%.[27]

Studies suggest MVA–BN might be less protective in the days immediately after the first vaccine dose than predicted from previous smallpox vaccine studies. In England, vaccine effectiveness 0–13 days after the first MVA–BN dose among GBMSM was –4% (95% CI –50 to 29) with no evidence of protection under any scenario in the sensitivity analysis.[25] Similarly, a French study[28] reported 12 (4%) breakthrough mpox infections among 276 vaccinated individuals, including ten within 5 days of vaccination. This has implications for the recommendation of MVA–BN for post-exposure prophylaxis. However, further studies are needed to assess whether post-exposure vaccination might modify disease severity in breakthrough cases. In children, the risk of secondary disease after exposure to a confirmed mpox case appears to be low. Following exposure to a staff member with confirmed mpox disease in four different educational settings, for example, we did not identify a single case among 340 or more exposed students and more than 100 exposed staff members during the 28-day follow-up period.[19] Mpox risk in household settings also appears to be very low, given that there was only one childhood case in a neonate with perinatally acquired infection during the current UK outbreak.[4,6] Reassuringly, our review of the global literature[29] found most children

to have mild, self-limiting illness, with very few hospitalisations and no deaths as of Sept 6, 2022. Overall, our experience with managing the current mpox outbreak has highlighted the importance of long-term investment in better surveillance, reporting, investigation, treatment, and prevention of neglected diseases, including mpox, in endemic countries, so that we have more robust data to develop evidence-based guidelines and inform national and international policy.

In conclusion, this is the first report of MVA–BN use in a small number of children. A single MVA–BN dose for post-exposure prophylaxis was well tolerated and induced robust antibody and cellular immune responses more than 3 months after vaccination. Our results need to be replicated in a larger cohort of unexposed children. Further studies are also needed to assess immune responses after two doses and duration of immune responses after vaccination in children.

References
1 Mitjà O, Ogoina D, Titanji BK, et al. Monkeypox. *Lancet* 2023; **401:** 60–74.
2 UK Health Security Agency. Monkeypox outbreak: epidemiological overview, 29 November 2022. GOV.UK, 2022. https://www.gov.uk/government/publications/monkeypox-outbreak-epidemiological-overview/monkeypox-outbreak-epidemiological-overview-29-november-2022 (accessed Feb 15, 2023).
3 UK Health Security Agency. Guidance: Monkeypox outbreak: vaccination strategy. GOV.UK, 2022. https://www.gov.uk/guidance/monkeypox-outbreak-vaccination-strategy (accessed Feb 15, 2023).
4 Ramnarayan P, Mitting R, Whittaker E, et al. Neonatal monkeypox virus infection. *N Engl J Med* 2022; **387:** 1618–20.
5 WHO. WHO Director-General declares the ongoing monkeypox outbreak a public health emergency of international concern. World Health Organization, 2022. https://www.who.int/europe/news/item/23-07-2022-who-director-general-declares-the-ongoing-monkeypox-outbreak-a-public-health-event-of-international-concern (accessed Feb 15, 2023).
6 UK Health Security Agency. Research and analysis: investigation into monkeypox outbreak in England: technical briefing 7. GOV.UK, 2022. https://www.gov.uk/government/publications/monkeypox-outbreak-technical-briefings/investigation-into-monkeypox-outbreak-in-england-technical-briefing-7 (accessed Feb 15, 2023).
7 Cohen JM, Bamford A, Eisen S, et al. Care of children exposed to monkeypox. *Lancet Reg Health Eur* 2022; **21:** 100514.
8 UK Health Security Agency. Guidance: high consequence infectious diseases (HCID). GOV.UK, 2023. https://www.gov.uk/guidance/high-consequence-infectious-diseases-hcid (accessed Feb 15, 2023).
9 UK Health Security Agency. Recommendations for the use of pre and post-exposure vaccination during a monkeypox incident. GOV.UK, 2022. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1100600/recommendations-for-pre-and-post-exposure-vaccination-during-a-monkeypox-incident-26-august-2022.pdf (accessed Feb 15, 2023).
10 Earl PL, Americo JL, Wyatt LS, et al. Rapid protection in a monkeypox model by a single injection of a replication-deficient vaccinia virus. *Proc Natl Acad Sci USA* 2008; **105:** 10889–94.
11 Pittman PR, Hahn M, Lee HS, et al. Phase 3 efficacy trial of modified vaccinia Ankara as a vaccine against smallpox. *N Engl J Med* 2019; **381:** 1897–908.
12 UK Health Security Agency. Green Book (Immunisation Against Infectious Diseases) Chapter 29: Smallpox and monkeypox. GOV.UK, 2022. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1106454/Green-Book-chapter-29_Smallpox-and-monkeypox_26September2022.pdf (accessed Feb 15, 2023).
13 Tameris MD, Hatherill M, Landry BS, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* 2013; **381:** 1021–28.
14 Ota MO, Odutola AA, Owiafe PK, et al. Immunogenicity of the tuberculosis vaccine MVA85A is reduced by coadministration with EPI vaccines in a randomized controlled trial in Gambian infants. *Sci Transl Med* 2011; **3:** 88ra56.
15 Afolabi MO, Tiono AB, Adetifa UJ, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in west African children and infants. *Mol Ther* 2016; **24:** 1470–77.
16 Otter AD, Jones S, Hicks B, et al. Analogous humoral antigen recognition between monkeypox-infected and smallpox-vaccinated individuals. *MedRxiv* 2022; published online Dec 26. https://doi.org/10.1101/2022.12.22.22283648 (preprint).
17 Wright J, Small N, Raynor P, et al. Cohort profile: the Born in Bradford multi-ethnic family cohort study. *Int J Epidemiol* 2013; **42:** 978–91.
18 Dowell AC, Haigh TA, Ryan GB, Turner JE, Long HM, Taylor GS. Cytotoxic CD4+ T-cells specific for EBV capsid antigen BORF1 are maintained in long-term latently infected healthy donors. *PLoS Pathog* 2021; **17:** e1010137.
19 Ladhani SN, Aiano F, Edwards DS, et al. Very low risk of monkeypox among staff and students after exposure to a confirmed case in educational settings, England, May to July 2022. *Euro Surveill* 2022; **27:** 2200734.
20 Greenberg RN, Kennedy JS. ACAM2000: a newly licensed cell culture-based live vaccinia smallpox vaccine. *Expert Opin Investig Drugs* 2008; **17:** 555–64.
21 García AD, Meseda CA, Mayer AE, Kumar A, Merchlinsky M, Weir JP. Characterization and use of mammalian-expressed vaccinia virus extracellular membrane proteins for quantification of the humoral immune response to smallpox vaccines. *Clin Vaccine Immunol* 2007; **14:** 1032–44.
22 Townsend MB, Keckler MS, Patel N, et al. Humoral immunity to smallpox vaccines and monkeypox virus challenge: proteomic assessment and clinical correlations. *J Virol* 2013; **87:** 900–11.
23 DeHaven BC, Gupta K, Isaacs SN. The vaccinia virus A56 protein: a multifunctional transmembrane glycoprotein that anchors two secreted viral proteins. *J Gen Virol* 2011; **92:** 1971–80.
24 Hammarlund E, Lewis MW, Hansen SG, et al. Duration of antiviral immunity after smallpox vaccination. *Nat Med* 2003; **9:** 1131–37.

25 Bertran MA, Andrews N, Davison C, et al. Effectiveness of one dose of MVA-BN smallpox vaccine against monkeypox in England using the case-coverage method. *MedRxIv* 2022; published online Dec 14. https://doi.org/10.1101/2022.12.13.22282654 (preprint).

26 Wolff Sagy Y, Zucker R, Hammerman A, et al. Real-world effectiveness of a single dose of mpox vaccine in males. *Nat Med* 2023; **29:** 748–52.

27 Payne ABRL, Ray LC, Kugeler KJ, et al. Incidence of monkeypox among unvaccinated persons compared with persons receiving ≥1 JYNNEOS vaccine dose—32 US jurisdictions, July 31–September 3, 2022. *MMWR Morb Mortal Wkly Rep* 2022; **71:** 1278–82.

28 Thy M, Peiffer-Smadja N, Mailhe M, et al. Breakthrough infections after post-exposure vaccination against monkeypox. *medRxiv* 2022; published online Aug 4. https://doi.org/10.1101/2022.08.03.22278233 (preprint).

29 Khalil A, Samara A, O'Brien P, Ladhani SN. Monkeypox in children: update on the current outbreak and need for better reporting. *Lancet Reg Health Eur* 2022; **22:** 100521.