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Clinical and Immunological Efficacy of MVA-BN vaccination against Mpox: results of the prospective AP-HP Monkeyvax study

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Abstract

Background.

MVA-BN vaccine is the only effective tool against mpox. It is recommended either as post-exposure prophylaxis (PEP), or pre-exposure prophylaxis (PrEP), with one dose for those vaccinated against smallpox during childhood (born < 1980 in France) and 2 doses otherwise. However, there are limited clinical data on vaccine effectiveness and a lack of standardized tools to monitor vaccine immunogenicity. Those are crucial to adjust current vaccination guidelines and ease vaccination monitoring globally. Our objective was to measure safety, failure rate, humoral and cellular immune response of MVA-BN vaccination in individuals at risk of mpox.

Method.

The AP-HP Monkeyvax study was a prospective multicentric cohort of individuals vaccinated with MVA-BN vaccine as PEP or PrEP. The primary objective was to estimate the vaccine failure rate, measured as symptomatic mpox confirmed by PCR anytime after PEP or 15 days after the end of primary vaccine schedule for PrEP. Secondary objectives included the evaluation of asymptomatic infection rate, safety, humoral and cellular immune responses, measured by vaccinia virus(VACV) neutralizing antibodies, MSD and Luminex Immuno-assays, and by INF-γ/ IL-2 ELISpot in CD4 + and CD8 + T cells. We compared MVA-BN immunogenicity between participants born before 1980 vaccinated with one dose and participants born ≥ 1980 vaccinated with 2 doses. We examined the correlations between neutralization and immune assays, and between neutralization and T cell response.

Results.

From July 2022 to October 2023, 164 participants were included, of whom 156 were analyzed: 110 vaccinated as PrEP and 46 as PEP. One symptomatic breakthrough case and one asymptomatic case occurred in the latter group. Higher and durable humoral responses were obtained among those vaccinated with one dose (previous smallpox vaccine during childhood) compared to those vaccinated with 2 doses of MVA-BN as primary vaccine schedule, who have sharp decline after 3 months to low levels at one year. Immuno-assays conducted with the MSD platform showed good correlation with neutralizing antibodies compared to Luminex. CD4 + and CD8 + T cell responses were robust and early in populations, with no correlate found with humoral response.

Discussion/conclusion.

In this prospective study, two cases of mpox were evidenced after 46 participants were vaccinated as PEP: one symptomatic and one asymptomatic. There were no mpox cases among those vaccinated as PrEP. Humoral immunogenicity declined sharply after 3 months, and reached a low level at 12 months. Our data

suggest that immunoassays can help monitor humoral response to MVA-BN vaccine when VACV neutralization is unavailable.

INTRODUCTION

Mpox is a zoonotic disease, identified in 1970, caused by the monkeypox virus (MPXV). It is endemic in Africa and has two clades: I and II. Since May 2022, mpox (clade II) has spread globally through sexual transmission, primarily among men who have sex with men (MSM), leading the WHO to declare a Public Health Emergency of International Concern (PHEIC) in July 2022 due to clade IIb and a second PHEIC in August 2024 due to clade I (1,2). The Modified Vaccinia Ankara Bavarian Nordic (MVA-BN), is a third-generation smallpox live vaccine (non-replicative in humans), authorized in 2013 in Europe (EMA) under the commercial name IMVANEX®, to prevent smallpox. The same vaccine (named JYNNEOS® in the USA) was approved in North America in 2019 by the FDA to prevent smallpox and mpox (3). These authorizations were based on: 1) a challenge trial conducted in macaques demonstrating 100% protection against MPXV infection after two MVA-BN injections, 2) a randomized, controlled, phase 3 trial, that assessed MVA-BN vaccine immunological efficacy against vaccinia, a second generation small pox vaccine (ACAM2000) (4,5). In France, as in the rest of the world, vaccine recommendations were quickly implemented in 2022. First, MVA-BN was recommended for post-exposure prophylaxis (PEP) in the 15 days following high-risk exposure, and then for pre-exposure prophylaxis (PrEP) for high-risk adults. Vaccine schedule was 1 dose sub-cutaneous (SC) for adults born before 1980 (because they had been vaccinated during childhood with 1st generation smallpox vaccine) and 2 doses, 28 days apart, for adults born after 1980. While no randomized control trial could be performed to demonstrate the efficacy of MVA-BN vaccine against mpox, observational studies have estimated vaccine effectiveness (VE) against symptomatic laboratory-confirmed mpox from 20.3% to 79.5% in post exposure prophylaxis (PEP), and approximately 80% in pre-exposure prophylaxis (PrEP). Most of these studies were based on registry or surveillance, and did not detect asymptomatic cases (1). Thus, VE could be over-estimated. Moreover, humoral immunological efficacy studies showed low or delayed response, which are discordant with clinical observational data(6). Meanwhile, a US observational cohort study found that mpox cases among fully vaccinated individuals occurred at a median of 266 days after vaccination(7). This discrepancy may be explained by good T cell immunogenicity, but comprehensive studies looking at both humoral and cellular immune response are lacking. Finally, there is need for standardized assays to measure vaccine response, especially in low- and middle- income countries (LMICs), because neutralization is resource-intensive and not scalable. We present here the results of the AP-HP Monkeyvax study, a prospective cohort of MVA-BN vaccinated adults with systematic testing, and with assessment of both humoral immune responses using neutralization, Meso-Scale and Luminex assays, up to one year post MVA-BN vaccination, and cellular immune responses during the first three months after vaccination.

METHODS

Study design, objectives and outcomes

The Monkeyvax study, sponsored by Assistance Publique – Hôpitaux de Paris, was a French national multicenter prospective cohort conducted in 13 participating centers in France in collaboration with the Inserm F-CRIN I-REIVAC network, designed to evaluate clinical and immunological efficacy of MVA-BN vaccine. The primary objective was the rate of vaccination failure, defined by the proportion of participants with symptoms suggestive of mpox and a positive PCR for mpox within 28 days of the first vaccine (for PEP), or between 14 days and 3 months after the 1st dose for people vaccinated on PrEP. Secondary objectives included asymptomatic infections, defined by positive PCR for mpox with no clinical symptoms. PCR were systematically performed in blood, urine, and oral swabs at 7, 14, 28, 43 days and 3, 6, and 12 months after the first dose of MVA-BN or at an additional visit scheduled for suggestive symptoms. Adverse reactions related to reactogenicity were collected according to a predefined list: for local reactions, pain, nodule, discoloration, hematoma, heat, erythema, swelling, induration, pruritus and rash, and for systemic reactions, myalgia, arthralgia, fatigue, chills, headache, nausea, fever, anorexia, lymphadenopathy. Severity was graded according to FDA (8). Our study also had immunological objectives, which included humoral immune response at each visit, and cellular immune response assessed at baseline (D0), between day 7 and 14 after the first dose (D10), and 3 months (M3) after the first dose.

The study protocol (No. EudraCT: 2022-002352-39) was conducted in accordance with the Declaration of Helsinki principles, the ICH Harmonised Tripartite Guideline for Good Clinical Practice (ICH E6), the French law governing research involving human subjects (known as ‘Loi Jardé’), and in full compliance with EU GDPR (General Data Protection Regulation) requirements. The study was approved by the Ethics Committee (CPP Ile de France III) and all participants gave written informed consent.

Study population

We included adults eligible for MVA-BN vaccination according to the French guidelines. In summary, PEP vaccination was indicated for individuals with direct skin-to-skin or mucosal contact and/or indirect contact on textile or surface and/or droplets exposure (presence without masks at less than 2 meters during at least 3 hours) with a PCR-confirmed mpox patient for less than 15 days (9). PrEP vaccination was indicated for individuals who self-identified as gay, bisexual or other MSM, or other individuals with multiple sexual partners; and health workers at high risk of exposure, laboratory personnel working with orthopoxviruses; clinical laboratory personnel performing diagnostic testing for monkeypox virus; and outbreak response team members (as designated by national public health authorities).

Humoral immune response

We used three techniques to assess humoral immune responses. The first technique evaluated anti-vaccinia virus (VACV) neutralizing antibody (nAb) titers by microneutralization using viral particles of the strain Western Reserve collected before the cytopathic effect and therefore composed mainly of extracellular viral forms. The second assessed IgG antibodies to five MPXV antigens (A29, A35R, B6R, E8L, M1R) and five VACV antigens (A27L, A33R, B5R, D8L, L1R) by ELISA assay using the multi-array electro-

chemiluminescent technology (MesoScale Diagnostics (MSD), Gaithersburg, MD). The assay was run in accordance with the manufacturer's protocol. The third technique was a Multiplexed Microsphere Suspension Array-Based Immunoassay performed on the Luminex platform and assessing IgG antibodies against seven MPXV antigens (A5L, A27L, A29L, A35R, B2R, H3L, M1R) and three VACV antigens (A27L, A33R, B5R). The assay was performed as described in the supplementary method.

Cellular immune response

T cell response was assessed *ex vivo*, by measuring IFN-γ and IL-2 production by specific CD4 + and CD8 + T cells, using the double enzymatic ELISpot technique following manufacturer's instructions (CTL Immunospot, Bonn Germany). Tested samples were collected at baseline (D0), between day 7 and 14 after the first dose (D10), and 3 months (M3) after the first dose. CD4 + T and CD8 + T cells were obtained from thawed peripheral blood mononuclear cell (PBMC) by a positive selection with a system MACS cell Separation using CD4 beads and LD columns (Miltenyi Biotec, Paris). Cells were resuspended in CTL medium enriched with 0.1µg/mL of anti-CD28. 100,000 cells/well were sensitized during 24h at 37°C and 5% CO2 with the Jynneos vaccine (Bavarian Nordic, Danemark) diluted at 1/500e, or 1 µg/ml of the Pan-Poxviridae Select PepMix pool, prepared as recommended by the JPT peptide technologies provider (Berlin, Germany), and consisting of 127 peptides derived from MPVX, Variola virus and Vaccinia virus.

Statistical analysis

We compared immune response between older participants born before 1980 (supposedly vaccinated against smallpox with VACV historical vaccine), who received one dose of MVA-BN, and younger participants, born after 1980, who received two doses. A complementary analysis also accounted for HIV status.

Analysis of neutralizing antibody assays: at each timepoint, we reported the geometric mean titers and their 95% confidence interval, the proportion of detectable neutralizing antibodies, as well as the proportion of sero-response, defined by participants who either became sero-positive for nAb, or showing a significant increase in titer defined as a pre-vaccination nAb titer ≥ 20 and at least a fourfold increase in post-vaccination titer. The proportions were presented with their Clopper-Pearson 95% confidence interval. Analysis of immune-assays: We presented immune-assays results in ratio. For the analysis with satured signal for MSD assay, we used the upper limit of detection (100,000). We presented the results using a ratio defined by the value of the signal by the mean signal + 2 standard deviations of true negatives. A ratio below < 0.7 was considered as negative and > 1.2 positive. Correlations with neutralizing antibody was assessed using Spearman Rank correlation at D0 and D28. We then selected antigens with a correlation > 0.4 at both time points for principal component analysis (PCA).

Cellular immunity: A response was considered positive if the number of spots in the stimulated wells was at least two-fold higher than the number of spots in the negative control, using a cutoff of 10 SFC/100,00 cells after background subtraction as previously detailed (10). The induction of a response following vaccination was set up as a positive response with at least a two-fold increase of cytokine-producing

specific CD4 + or CD8 + T cells at 2 months after the second vaccination compared to D0. Correlations with vaccinia virus antibody titers up to M3 were obtained with Spearman correlations with p-values adjusted for multiplicity. Statistical analyses were performed with SAS 9.4.

RESULTS

Characteristics of the cohort

Between July 2022 and October 2023, 164 participants were enrolled, of whom 156 were analyzed (46 in the PEP group and 110 in the PrEP group) (Fig. 1). The median follow-up was 11.5 months (3.0 months in the PEP group and 11.7 months in the PrEP group). Male represented 96.8% of the population (151/156), median age was 36 years (IQR: 29–48), 30.8% (48/156) were born before 1980. People living with HIV (PLWH) represented 26.3% of the population: 13.0% in the PEP group and 31.8% in the PrEP group (Table 1). In the PEP population, 52% of participants reported exposure through a sexual partner, and 20% through fomites. The median number of days between exposure and vaccination was 7 (4–12). The distribution of vaccine primary schedule by birth dates are presented in supplementary table 1.

Table 1
Characteristics of the cohort at inclusion

	Population			
	PEP (N = 46)	PrEP (N = 110)	Total (N = 156)	P-value
Male, n (%)	41 (89%)	110 (100%)	151 (97%)	0.0019[1]
Median Age, y (IQR)	36 (30, 41)	36 (29, 54)	36 (29, 48)	0.542[2]
Born < 1980	10 (22%)	38 (35%)	48 (31%)	0.11[3]
Born ≥ 1980	36 (78%)	72 (65%)	108 (69%)	
Immunosuppression (other than HIV), n (%)	1 (2%)	1 (0.9%)	2 (1.3%)	0.50[1]
Living with HIV	6 (14%)	35 (32%)	41 (26%)	0.026[1]
Undetectable HIV viral load, n (%)	5 (98%) 1 NA	29 (83%)	34 (83%) 1 NA	1[1]
CD4 T-helper cell count (cells/µl), median (IQR)	646 (585, 744) 1 NA	741 (590, 810) 1 NA	739 (585, 810) 2 NA	0.59[2]
<200	0 (0%)	1 (3%)	1 (3%)	1[1]
200–350	0 (0%)	2 (6%)	2 (5%)	
350–500	0 (0%)	4 (12%)	4 (10%)	
>=500	5 (100%)	27 (79%)	3 (82%)	

PEP vs PrEP P-value [1]Fisher Exact p-value; [2]Kruskal-Wallis p-value; [3]Chi-Square p-value;

Clinical outcomes

One case of vaccine breakthrough according to our primary outcome was reported: a participant aged 28 years and living with well-controlled HIV infection (744 CD4/µL, undetectable viral load) who developed symptoms 3 days after PEP vaccination and 7 days after exposure, with a positive MPXV PCR result at D5 in skin and anal sample (Table 5). Subsequent analysis at the reference laboratory revealed a positive MPXV PCR at D0 in urine sample (table 5). An additional participant had an asymptomatic infection, confirmed by a medical clinical assessment: a 30-year-old male living with HIV, with 873 CD4/µL and undetectable viral load, who received PEP vaccination 6 days after exposure. A positive PCR result at D7 in the throat was obtained at the reference laboratory (table 5). Four other participants reported symptoms

suggestive of mpox, but none of them was clinically diagnosed with mpox, and all PCRs were negative (see Table 2).

Table 2
Description of suspected and mpox cases

Parameters	Participants					
	#1	#2	#3	#4	#5	#6
Age, years	28	30	27	52	31	20
Sex	Male	Male	Male	Male	Male	Male
Living with HIV	Yes	Yes	No	No	No	No
Plasma HIV viral load	< 20 cp/mL	< 20 cp/mL	NA	NA	NA	NA
$CD4/mm^3$	744	873	NA	NA	NA	NA
Indication	PEP	PEP	PEP	PEP	PrEP	PrEP
Number of doses	1	1	1	1	2	2
Time from exposure to vaccination	5	5	NA	NA	NA	NA
Symptoms	Headache, Lymphadenopathy, Myalgia, Asthenia, Cutaneous and mucosal lesions (oral and genital mucosa)	None	Influenza like illness Skin rash (face/neck)	Skin rash (not specified)	Angina Fatigue adenopathy	Angina and genital ulceration
Time from vaccination to symptoms, days	2	NA	2	2	3	35
Positive samples	Urine* Skin Anal**	Throat	None	None	None	None
Time from vaccination to positive samples, days	0	7	NA	NA	NA	NA

*Positive at the reference laboratory but not at investigation site

**Skin and anal samples positive at investigation site at day 5 after vaccination

Reactogenicity

We collected reactogenicity data following the first and second doses for 154/156 and 118/123 participants, respectively. Overall, 78.6% (121/154) experienced local side effects (84% mild in severity), and 23.4% (36/154) reported systemic side effects (61% mild in severity). Adverse events occurred more frequently after the first dose, with 3 severe AEs: 1 fever (39.0 to 40°C) for 1 day; 1 nodule for 6 days; 1 local pain for 3 days (see Fig. 2).

Humoral immune response

Neutralizing assays (Table 3 and Fig. 3)

Table 3
Humoral Response

		D0	D14	D28	D43	M3	M6	M12
Born < 1980, 1 dose								
Neutralizing antibodies titers	N	29	10	31	11	30	29	29
	GMT (95% CI)	28.6 [20.2 ; 40.6]	105.6 [45.2 ; 246.8]	80.0 [55.2 ; 116.0]	75.1 [33.1 ; 170.3]	50.4 [35.1 ; 72.4]	41.0 [27.9 ; 60.1]	63.0 [41.7 ; 95.2]
Seroconverters, ≥ 2 fold-rise*	n/N, %, (95% CI)	-	9/9, 100% [66.4 ; 100]	24/29, 82.8% [64.2 ; 94.2]		14/28, 50.0% [30.6 ; 69.4]		
Seroconverters, ≥ 4 fold-rise*	n/N, %, (95% CI)	-	8/9, 88.9% [51.8 ; 99.7]	17/29, 58.6 [38.9 ; 76.5]		7/28, 25.0%, [10.7 ; 44.9]		
Detectable nAb titer (≥ 20)	n/N, % (95% CI)	21/29, 72.4% [52.8 ; 87.3]	10/10, 100% [69.2 ; 100%]	29/31, 93.5% [78.6 ; 99.2]		26/30, 86.7% [69.3 ; 96.2]		
Born ≥ 1980, 2 doses								
Neutralizing antibodies titers	N	80	20	100	32	94	75	73
	GMT (95% CI)	11.2 [10.4 ;12.1]	24.6 [16.8 ; 36.0]	20.7 [18.3 ; 23.4]	59.1 [43.9 ; 79.5]	42.1 [35.6 ; 49.9]	26.6 [22.2 ; 31.9]	43.2 [31.6 ; 59.0]
Seroconverters, ≥ 2 fold-rise*	n/N, %, (95% CI)		14/19 ; 73.7% [48.8 ; 90.9]	54/75 ; 72.0% [60.4 ; 81.8]	16/18 ; 88.9% [65.3 ; 98.6]	66/74 ; 91.9% [83.2 ; 97.0]		
Seroconverters, ≥ 4 fold-rise*	n/N, %, (95% CI)		13/20 ; 68.4% [43.4 ; 87.4]	54/75 ; 72.0% [60.4 ; 81.8]	17/18 ; 94.4% [72.7 ; 99.9]	66/94 ; 89.2% [79.8 ; 95.2]		
Detectable nAb titer (≥ 20)	n/N, % (95% CI)	10/80, 12.5% [6.2; 21.8]	10/10, 100% [69.2 ; 100%]	29/31, 93.5% [78.6 ; 99.2]		26/30, 86.7% [69.3 ; 96.2]		

* from baseline (D0) or a titer < 20 at D0 followed by a titer ≥ 20

Before vaccination with MVA-BN vaccine, the proportion of detectable nAb was 72.4% (21/29) among participants born < 1980 and vaccinated with one dose (GMT: 28.6, $CI_{0.95}$= [20.2–40.6]), compared with 12.5% (10/80) among those born ≥ 1980 and vaccinated with 2 doses (GMT: 11.2, $CI_{0.95}$= [10.4–12.1]). At D28 (i.e. 28 days after the first injection of MVA-BN vaccine), among those born < 1980 with one injection, 93.5% (29/31) had detectable neutralizing antibodies (GMT: 80.0, $CI_{0.95}$= [55.2–116.0]), compared with 72% (72/100) of those born ≥ 1980 with two injections (GMT: 20.7, $CI_{0.95}$= [18.3–23.4]). In the latter group, at D43 (28 days after the second injection), 94% (30/32) had detectable neutralizing antibodies (GMT: 59.1, $CI_{0.95}$= [43.9–79.5]). Finally, at M12, among those born < 1980 who received one MVA-BN injection, 93.1% (27/29) had sustained detectable neutralizing antibodies (GMT: 63.0, $CI_{0.95}$= [41.7–95.2]), while 79.5% (58/73) of participants born ≥ 1980 who received two MVA-BN injections had detectable neutralizing antibodies (GMT: 43.2, $CI_{0.95}$= [31.6–59.0]). Exploratory sub-analysis on early response showed that at D14, among those born < 1980 with one injection, 100% (10/10) of participants had detectable nAb response (GMT: 105.6, $CI_{0.95}$= [45.2–246.8]), compared with 80.0% (16/20) of participants born ≥ 1980 with two injections (GMT: 24.6, $CI_{0.95}$= [16.8–36.0]).

Seroresponse

We also looked at participants who either became sero-positive for nAb, or showing a significant increase in titer defined as a pre-vaccination nAb titer ≥ 20 and at least a fourfold increase in post-vaccination titer. At D14, among those born < 1980 with one injection, 8/9 (89%) seroconverted vs 13/19 (68%) among participants born ≥ 1980 with two injections, and respectively 9/9 (100%) vs 14/19 (74%) using a twofold increase definition. At D28, among those born < 1980 with one injection, 17/29 (59%) seroconverted vs 54/75 (72%) among participants born ≥ 1980 with two injections, and respectively 24/29 (83%) vs 54/75 (72%) using a twofold increase definition. At D43, among participants born ≥ 1980 who received two MVA-BN injections, seroconversion was 16/18 (89%) using fourfold increase and 17/18 (94%) using twofold increase.

Characteristics of participants living with HIV (PLWH)

Our cohort study had a total of 41 PLWH: 6/41 (14.6%) in the PEP cohort, and 35/110 (31.8%) in the PrEP cohort. Viral load was undetectable in 33/40 (82.5%), median CD4 cell count was 739/µL (IQR = [585, 810]). Of note, one participant was diagnosed with HIV at inclusion (Table 1). No difference was observed when comparing humoral immune response between PLWH and the rest of the cohort: the proportion of detectable nAb at D28 and M12 for PLWH was respectively 13/14 (93%, GMT: 84.1, $CI_{0.95}$= [46.3– 152.5]) and 13/14 (92.9%, GMT: 72.5, $CI_{0.95}$= [41.3–127.2]) among those born < 1980 with one injection, and 9/14 (64.3%, GMT: 21.0, $CI_{0.95}$= [13.0–34.1]) and 13/17 (76.5%, GMT: 30.1, $CI_{0.95}$= [17.9–50.6]) among those born ≥ 1980 after their first injection (Supplementary Fig. 1).

Immunoassays

IgG titers: antibody ratios by antigens are presented in Fig. 4. The IgG titers measured by MSD assay yielded higher ratios than the Luminex assay: among the participants born < 1980 and vaccinated with one

dose, all antigens tested by MSD yield a ratio > 1.2, while only 4/10 antigens tested by Luminex yield a ratio > 1.2: MPXV A35, MPXV H3, MPXV VACV A33R, VACV B5. Among participants born ≥ 1980, IgG titers were lower: 8/10 yield ratio > 1.2 with the MSD platform (all except MPXV A 29L and VACV A27L), and only 1/10 using the Luminex platform (VACV B5).

Correlation with neutralizing antibodies

Using Spearman's correlation (Fig. 3), we eliminated 8 antigens which poorly correlated with neutralizing antibodies at D0 and D28. The 12 remaining antigens were analysed by PCA and represented on biplots (Supplementary Fig. 2). The pre-vaccination (D0) vaccinia virus nAb titer was highly correlated with the ELISA variables, and almost uncorrelated with Luminex. According to these measures of humoral immunity, populations defined by age (born < 1980 or ≥ 1980) had similar results and tended to cluster together. After vaccination (D28, M3, M6), the nAb titer remained uncorrelated with most of the Luminex variables but was correlated with VACV B5R and MPXV_B6R (MSD).

Cellular Immunogenicity

We evaluated the cellular immune response by IFN-γ/IL-2 ELISpot assay in 16 participants (2 PEP, 14 PrEP): 8 in participants born < 1980 vaccinated with one dose (including 1 PEP), and 8 in participants born ≥ 1980 vaccinated with 2 doses (including 1 PEP). First, CD4 + and CD8 + T cells were stimulated with a pan-Poxviridae peptide pool (from JPT Peptide Technologies), as previously described by others (11–13). We obtained very weak IFN-γ responses at M3 with this commercial peptide pool: 1/15 positive participant (6.7%) for CD4 + and 4/15 (25%) for CD8 + T cells, with median response levels of 1 [0 to 11] SFC/100,000 CD4 + T cells and 4 [0 to 132] SFC/100,000 CD8 + T cells. In previous studies using this peptide pool, the positivity threshold chosen was lower than ours (8–9), or not specified (10), reducing the test specificity (7). Therefore, we decided to evaluate the MVA-BN vaccine itself, as an *ex vivo* stimulating agent. Different vaccine dilutions were tested on CD4 + T cells, CD8 + T cells or total PBMC from two vaccinated healthy subjects. The 1:500 final dilution of MVA-BN in ELISpot wells was retained and validated in one vaccinated subject from the cohort and a pre-infected mpox subject. Assay specificity was checked on T-cells from 4 individuals, born after 2000 and free of any Poxviridae infection or vaccination, and their responses were below the positivity threshold.

Using a threshold of 10 spots/100,000 cells, we found similar high response rate against the MVA-BN vaccinal strain regarding INF-γ in CD4 + and CD8 + T cells (Fig. 6 and Table 4), respectively 9/14 (64.2%) and 11/14 (78.6%) at D10, and 12/15 (80.0%) and 13/15(86.7%) at M3, with median response levels at sM3 of 21.3 [2 to 159] SFC/100,000 CD4 + T cells, and 27.7 [1.3 to 660] SFC/100,000 CD8 + T cells. IL-2 response range was higher in CD4 + T cell compared to CD8+, respectively 10/14 (71.4%) vs 3/14 (21.4%) at D10 and 14/15 (93.3%) vs 6/15(40.0%) at M3, with median response levels of 25.7 [1.3 to 79.3] SFC/100,000 CD4 + T cells and 6.7 [0 to 159.3] SFC/100,000 CD8 + T cells. Co-secretion of IL-2 and INF-γ in CD4 + compared to CD8 + T cells was 4/14 (28.6%) vs 3/14 (21.2%) at D10 and 7/15 (46.7%) vs 6/15 (40.0%) at M3. Three months after vaccination, all the 15 tested individuals had at least a significant CD4 + or a CD8 + T cell response, secreting IFN-γ and/or IL-2. With this limited sample size, we did not find any differences when comparing participants born before 1980 vaccinated with one dose, and participants

born after 1980 vaccinated with 2 doses, except for IFN-γ secreting CD8 + T cells at M3 (p = 0.037). Finally, looking at correlation between humoral and cellular immunogenicity, using Spearman correlations, we did not find significant correlations of responses after D0 and nAb titers (Supplementary Fig. 3).

Table 4
Cellular immune response* in participants born < 1980 vaccinated with one dose of MVA-BN and participants born ≥ 1980 vaccinated with two doses.

		Proportion of responders (n/N, %)		
T-cell	Cytokines	D_0	D_{10}	M_3
Born < 1980, 1 dose				
CD4	IFNγ	2/8 (25%)	4/6 (67%)	7/8 (88%)
	IL-2	2/8 (25%)	4/6 (67%)	8/8 (100%)
	IFNγ + IL2	1/8 (13%)	3/6 (50%)	4/8 (50%)
CD8	IFNγ	2/8 (25%)	5/6 (83%)	6/8 (75%)
	IL-2	0/8 (0%)	2/6 (33%)	1/8 (13%)
	IFNγ + IL-2	0/8 (0%)	2/6 (33%)	1/8 (13%)
Born ≥ 1980, 2 doses				
CD4	IFNγ	0/8 (0%)	5/8 (63%)	5/7 (71%)
	IL-2	0/8 (0%)	6/8 (75%)	6/7 (86%)
	IFNγ + IL-2	0/8 (0%)	1/8 (13%)	3/7 (43%)
CD8	IFNγ	1/8 (13%)	6/8 (75%)	7/7 (100%)
	IL2	0/8 (0%)	1/8 (13%)	5/7 (71%)
	IFNγ + IL-2	0/8 (0%)	1/8 (13%)	5/7 (71%)

* Defined by the participants with the numbers of spot-forming cells ≥ 2-fold higher than the number of spots in the negative control, using a cut-off of 10 SFC/100,000 cells (after negative control subtraction)

DISCUSSION

We present here the results of a MVA-BN vaccinated prospective cohort, with detailed clinical events, and comprehensive immune-monitoring assays. The safety data are reassuring and confirm previous findings of phase 3 trials showing a good safety profile (14). We found one participant with a virological confirmed symptomatic mpox among 46 participants vaccinated as PEP (2.1%). Interestingly, we also detected an asymptomatic case post-PEP. Our failure rate is within the range of 4%, reported by Thy *et al.* (15). Of note, PEP recommendations were set based on previous estimates of incubation period from 7 to 15 days, but recent modelling and contact-tracing studies of the 2022 outbreak have revised median incubation period

to be around 7 days (16–18). Most studies on MVA-BN VE are based on registry data and surveillance, which do not measure asymptomatic cases. Therefore, our results suggest that PEP VE could prevent symptomatic mpox, but perhaps not asymptomatic mpox with consequences on virus transmission.

Looking at humoral response, our results confirm those of previous studies that showed a better response among participants born < 1980 vaccinated with one dose, compared with those born ≥ 1980 vaccinated with two doses, and low level at early timepoints. Our study confirms that MVA-BN can trigger an anamnestic response in those vaccinated against smallpox in their childhood, which is stronger and more durable than in those primarily vaccinated with MVA-BN. We observed a sharp decline after 3 months, reaching a low level of neutralizing antibodies one year after vaccination among participants with MVA-BN as primary vaccine regimen. These are consistent with other publications, who found either undetectable or low level of humoral response (4,19–23). Unfortunately, the exact clinical significance cannot be inferred due to the lack of correlate of protection. These results confirm a waning immunity, which is corroborated with clinical data from the Guagliardo *et al.*, who studied mpox cases post vaccination, and found a median of 266 days between mpox onset and the second dose of MVA-BN vaccine (7). We also compared two commercially available immunoassays with VACV neutralization. Our aim was to evaluate these assays in order to study vaccine response. Some studies have shown the capability of MSD and Luminex assay to detect previous infectious and differentiate infected from vaccinated, but none compared these with neutralizing antibodies (20,24,25). We found that MSD immuno-assay had better signal/noise results, with higher ratio compared with Luminex. Indeed, results of Luminex immuno-assays showed that 7/10 antigens have ratio lower than 1. Looking at the raw results of Luminex immuno assays reported by Jones *et al.*, and ours, we found lower signal than reported. This can be explained by different outputs: in Jones *et al.*, immuno-assays were presented using arbitrary units, meaning they did not control for background noise. We choose a more conservative outputs by normalizing the signal by background noise (24). Nevertheless, using PCA, we found good correlation between neutralizating antibodies and MSD vaccinia B5R, A33R antigens and MPXV B6R, A35R antigens, as well as Luminex vaccinia B5 and MPXV H3 antigens. Analysis of cellular immune responses showed robust cellular immunity after MVA-BN vaccination, in line with findings reported by Mazzotta *et al.* and Matusali *et al.* No significant differences were observed according to the primary vaccination schedule, unlike what was observed for humoral immune responses (20,24). Early cellular response, measured 10 days post vaccination, may play a role in the observed clinical efficacy of PEP exposure (6). With a limited sample size, we were not able to see a correlation between humoral and cellular response, which is suggested by Drennan *et al.* and Cohn *et al.* (26,27).

Our study has some limitations: The sample size was relatively small with a loss to follow-up in the PEP population during the course of the study, which limited the possibility of statistical comparison between vaccination schedule and the interpretation of T cell response. We did not assess the long term cellular response to see whether it decreases as well as the humoral response. For the humoral assessment, we did not use MPXV neutralization to ensure the robustness of our results, since MPXV neutralization is not yet widely standardized and can produce highly variables results. Moreover, comparative studies have shown that MPXV and VACV neutralization are highly similar.

Our study has also strengths: we provide a rigorous comparison of multiple humoral assays to measure vaccine immunogenicity. Our results suggest that the MSD immunoassay can help monitor the immune response to the MVA-BN vaccine, which may be useful when seroneutralisation is not available. Luminex assay, although more affordable and more standardized, can also be used but the interpretation of the results (signal/noise ratio) needs improvement. Few studies have been able to directly compare humoral and cellular immunity. Using the vaccine itself after a CD4 magnetic enrichment, we optimized the analytical conditions to monitor specific cellular immunity induced after MVA-BN vaccination. We showed that MVA-BN vaccine was able to provide early and robust T cell response, which may explain the apparent discrepancy between humoral studies showing low and slow humoral response and observational studies showing good vaccine effectiveness, even given as PEP.

To conclude, we provide additional evidence of rapidly decreasing humoral response among persons with MVA-BN as primary vaccine schedule, a strong initial cellular response, and evidence that immuno-assays can be used to provide accessible, standardized and affordable tools to assess vaccine immunogenicity, especially in LMICs. The decrease in humoral response, evidenced by our study, together with data from an animal model establishing the important role in disease protection, was considered by the French National Immunization Technical Advisory Group (Haute Autorité de Santé) to recommend a booster dose (28,29).

Declarations

Competing interests

LBL has received personal fees as a speaker or consultant from Sanofi and Pfizer outside this work

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Figures

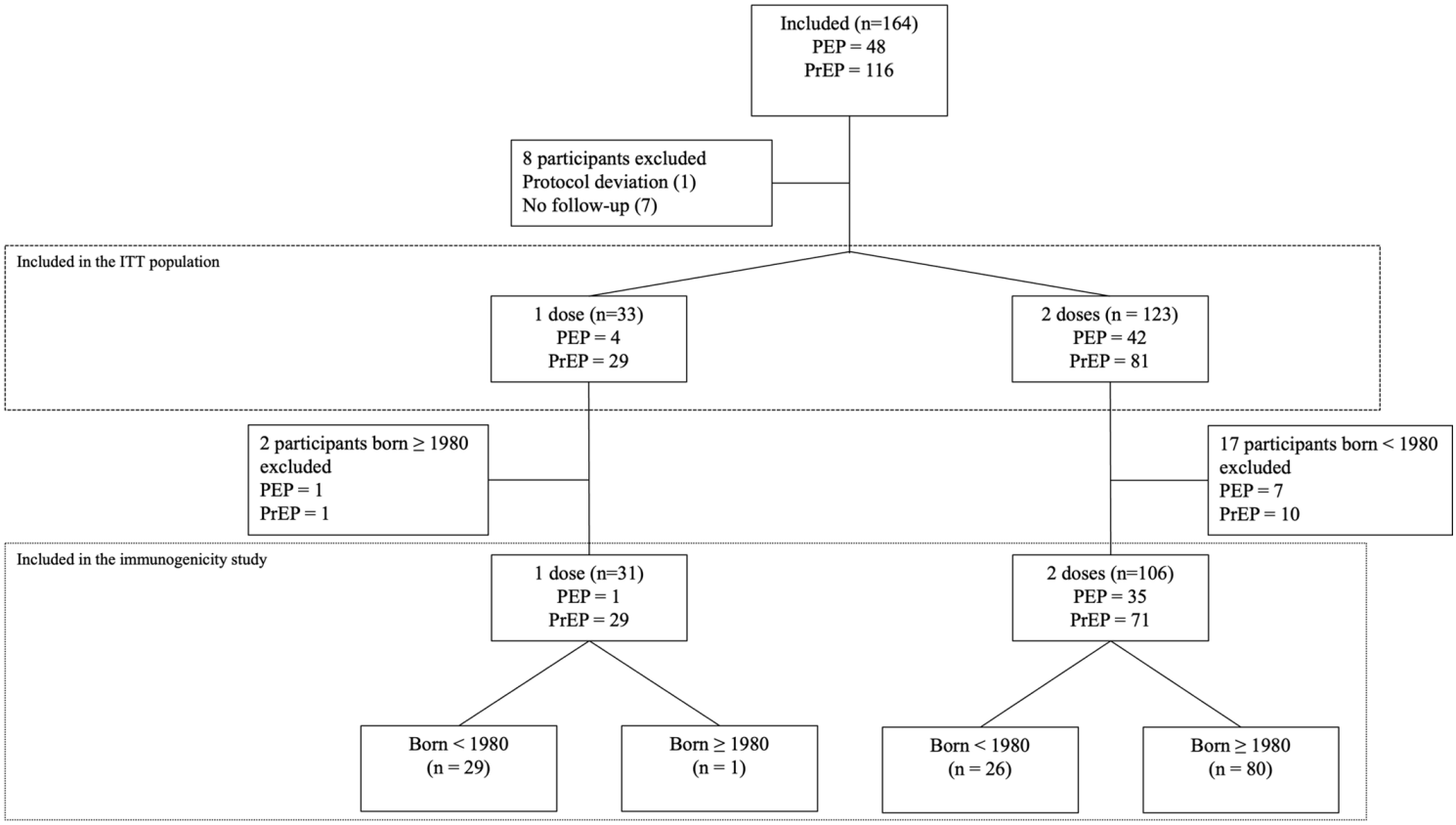


Figure 1

Flow chart. The ITT population is analysed for the reactogenicity, while the immunogenicity study includes all participants with at least one result after vaccination. PEP: post-exposure prophylaxis. PrEP: pre-exposure prophylaxis. ITT : Intention-to-treat.

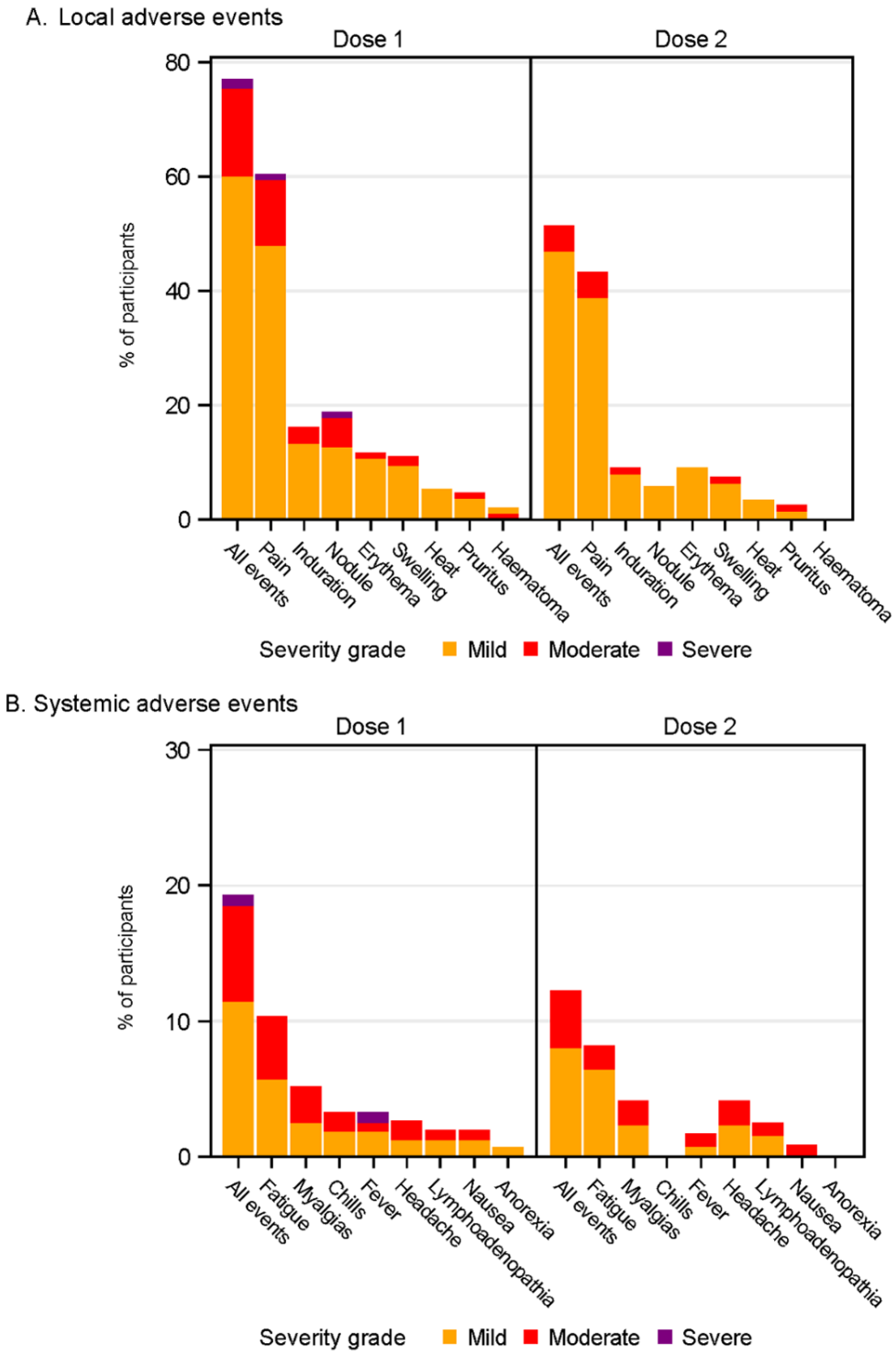


Figure 2

Reactogenicity. The bar charts represent the proportions of participants with local adverse events (2A) and systemic adverse events (2B). Mild, moderate and severe adverse events are represented in orange, red and purple, respectively.

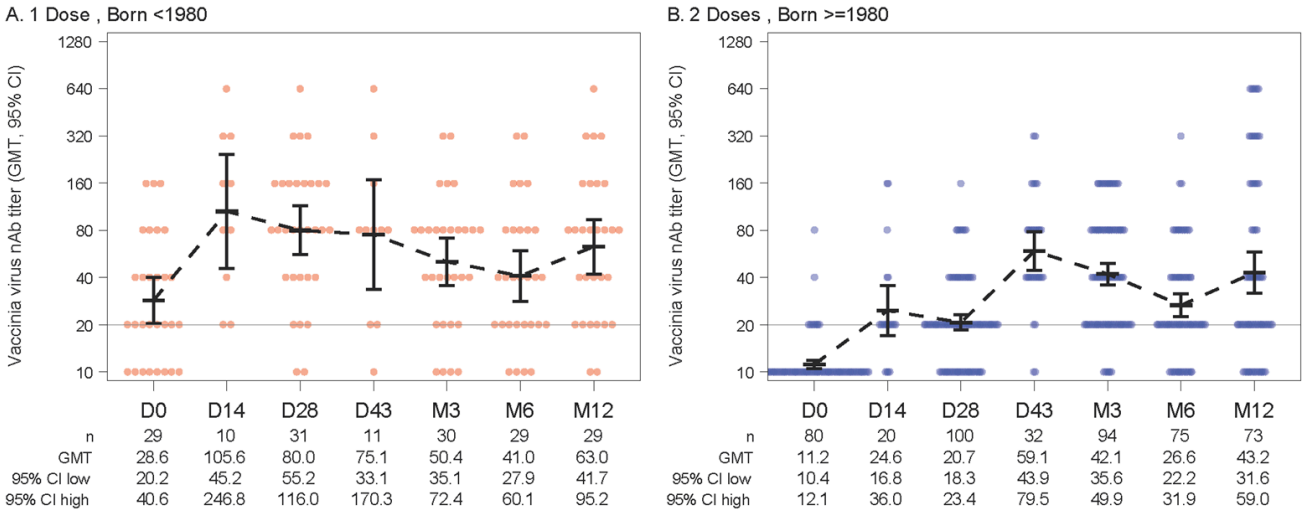


Figure 3

Vaccinia neutralizing antibodies titers. The graphs represents the GMTs of vaccinia neutralizing antibodies dilution titers according to primary vaccine regimen before vaccination (D0), at 14 days (D14), 28 days (D28), 43 days (D43), 3 months (M3), 6 months (M6) and 12 months (M12) after the first dose. nAb: neutralizing antibodies. GMT: Geometric mean titers. 95%CI : 95% confidence interval.

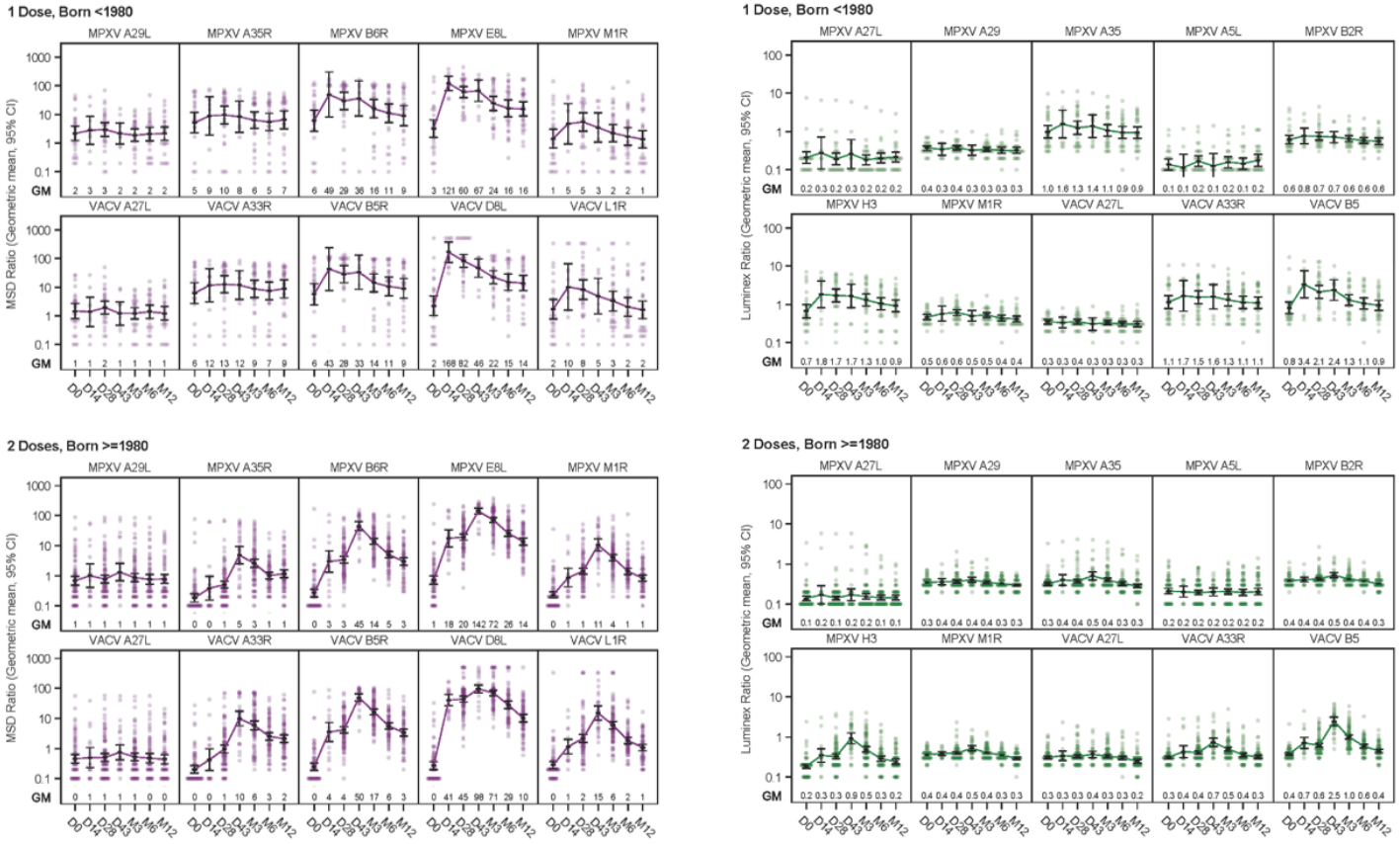


Figure 4

IgG titers in vaccinated individuals over time since vaccination, as measured by the MSD (in purple) and the Luminex Assay (in green), according to primary vaccine regimen schedule: the first line is the results for those born < 1980 who received 1 dose of MVA-BN. The second line is the results for those born ≥ 1980 who received 2 doses of MVA-BN. X axis display the timepoint : before vaccination (D0), at 14 days (D14), 28 days (D28), 43 days (D43), 3 months (M3), 6 months (M6) and 12 months (M12) after the first dose. Ratio represent the ratio of signal divided by the value of the signal by the mean signal + 2 standard deviations of the true negatives. Each dot represents a participant. Dashes showed the geometric mean. Whiskers represent the 95% confidence interval. The values of geometric means are noted at the bottom of each graph. GM : geometric mean.

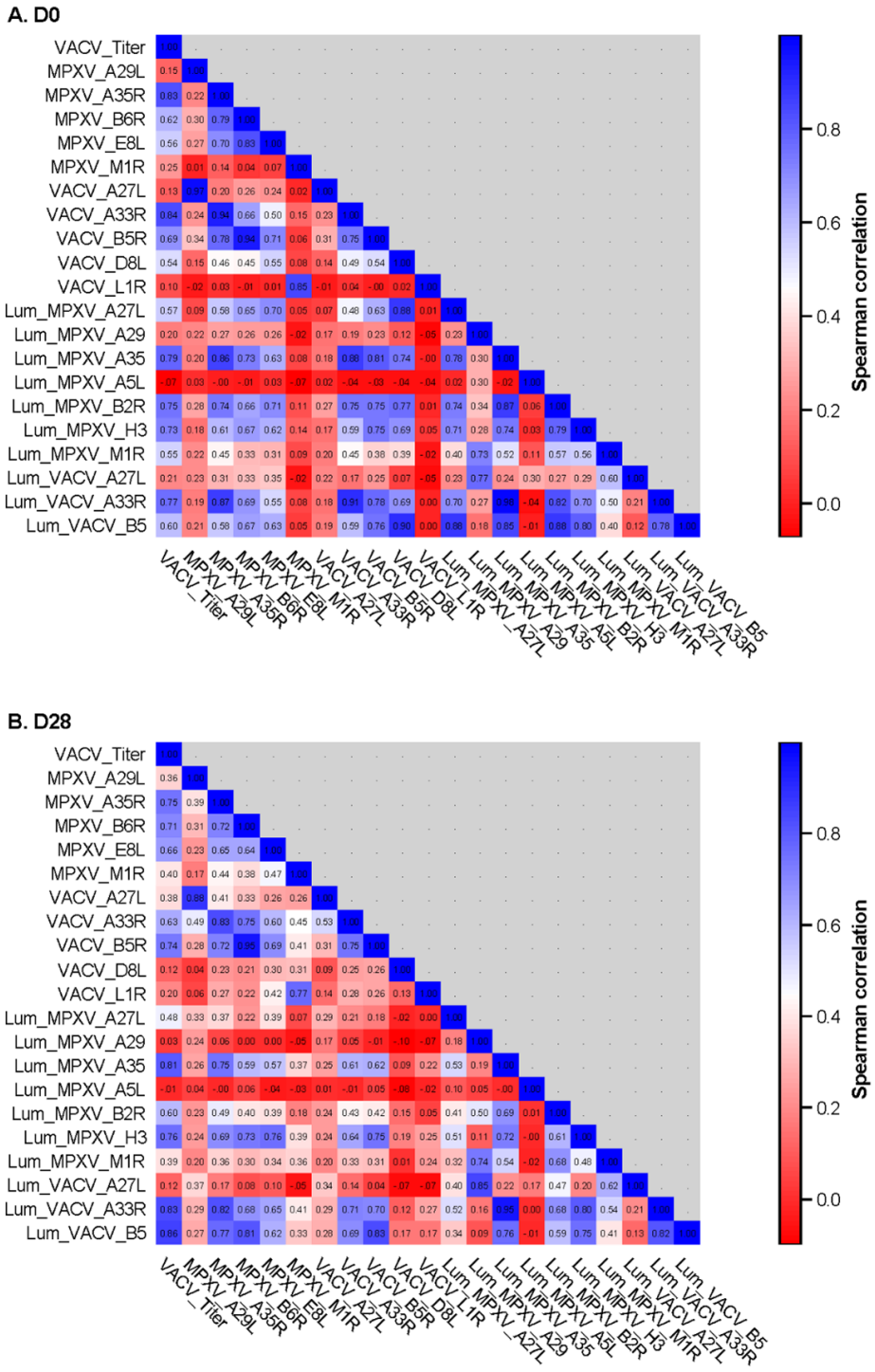


Figure 5

Correlation among humoral assays before vaccination (D0, participants with complete data n=122) and after one dose of MVA-BN vaccine (D28, n=148). Non-parametric Spearman correlations heatmap between each parameter (nAb titer: VAC_titer, MSD Assay: MPXV_A29L to VACV_L1R, Luminex Assay: Lum_MPXV_A29 to Lum_VACV_B5). Correlations are reported and their scale is shown on the right of the matrix, darker blue color indicating stronger correlations.

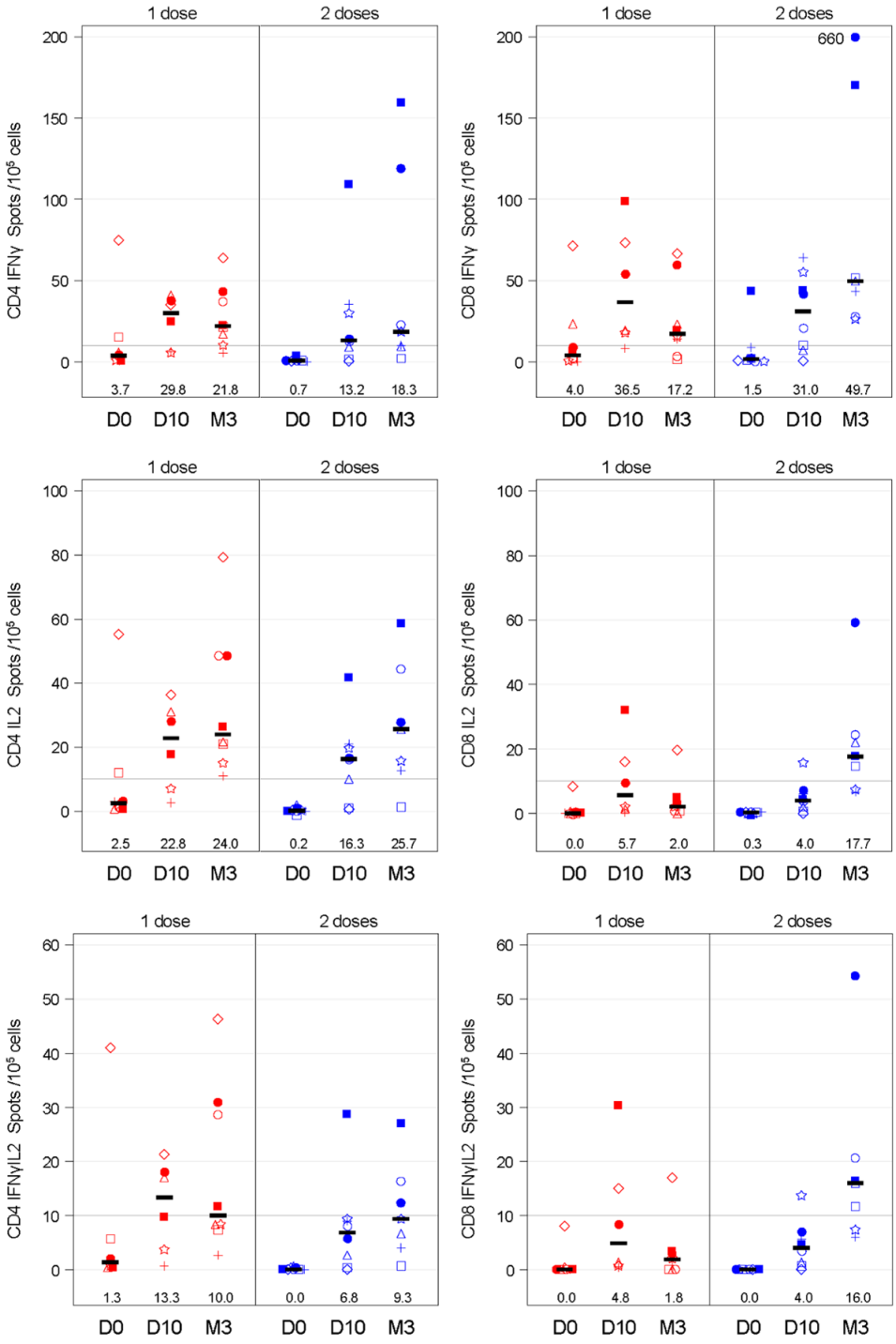


Figure 6

Cellular response

IFN-γ/IL-2 ELISpot assay in per-protocol populations (born < 1980 who received 1 dose n=8, born ≥1980 who received 2 doses n=8) after stimulation with MVA-BN vaccine antigens. The production of IFN-γ, IL-2 and IFN-γ IL-2 by CD4+ and CD8+ cells was measured by counting the number of spots in ELISpot plates

per 10^5 cells. The values for each subject (after negative control subtraction) are indicated by a specific symbol (the same symbol is used for the same subject throughout the graphs). Dashes and numbers indicate medians.

Supplementary Files

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- Supplementaryappendix.docx