Randomized clinical trial examining safety and immunogenicity of heterologous prime-boost Ebola vaccines, Ad26.ZEBOV and MVA-BN-Filo: 12-month data from Uganda and Tanzania

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Brief Summary: This Phase 1 study demonstrates that heterologous Ad26.ZEBOV, MVA-BN-Filo 2-dose vaccination against Ebola with a 28 and 56 day dosing interval is well tolerated and immunogenic in healthy African adult volunteers.

Abstract word limit: 200 words; **word count:** 200 words

Abstract

BACKGROUND: Ebola vaccine development was accelerated in response to the 2014 Ebola virus outbreak. This Phase 1 study (VAC52150EBL1004) assessed safety, tolerability, and immunogenicity of heterologous prime-boost Ad26.ZEBOV, MVA-BN-Filo vaccination regimens in the Lake Victoria Basin of Tanzania and Uganda in mid-level altitude, malarial-endemic settings.

METHODS: Healthy volunteers aged 18–50 years from Tanzania (n=25) and Uganda (n=47) were randomized to receive placebo or active vaccination with Ad26.ZEBOV or MVA-BN-Filo (prime vaccination), followed by MVA-BN-Filo or Ad26.ZEBOV, respectively (boost vaccination), with intervals of 28 or 56 days.

RESULTS: 72 adults were randomized to receive vaccination (N=60) or placebo (N=12). No vaccine-related serious adverse events (AEs) were reported. The most frequent solicited local and systemic AEs were injection site pain (frequency 70%, 66% and 42% per dose for MVA-BN-Filo, Ad26.ZEBOV and placebo, respectively) and headache (57%, 56% and 46%, respectively). AE patterns were similar among regimens. At 21 days post boost, 100% of volunteers demonstrated binding antibody responses against EBOV glycoprotein and 87–100% demonstrated neutralizing antibody responses. Ad26.ZEBOV priming induced more robust initial binding antibody and cellular responses than MVA-BN-Filo priming.

CONCLUSIONS: Heterologous prime-boost vaccination with Ad26.ZEBOV and MVA-BN-Filo against Ebola is well tolerated and immunogenic in healthy volunteers.

CLINICAL TRIAL NUMBER: NCT02376400

Keywords: Ebola vaccine, heterologous prime-boost, Ad26.ZEBOV, MVA-BN-Filo, safety and immunogenicity

Introduction

Ebola virus disease is highly contagious and severe, with a high fatality rate [1, 2]. The 2014 Zaire Ebolavirus outbreak in West Africa received extensive global attention because of the large number of cases (>28,600) and deaths (>11,000), and potential for further international spread [3].

Smaller outbreaks have occurred repeatedly in Central and East Africa since Ebola was first described in 1976 [4, 5]. In Uganda, five outbreaks of Ebola virus occurred between 2000 and 2012, with 606 suspected cases and 283 deaths (fatality rate: 47%) [6, 7]. More recent cases have been reported in the Democratic Republic of Congo (2017 and 2018) [8–10].

As a response to the 2013–2016 outbreak, efforts to develop Ebola vaccines were accelerated, with a variety of candidate vaccines under investigation using platforms including DNA, recombinant or subunit proteins, virus-like particles and recombinant viral vectors [11]. Heterologous prime-boost vaccination with Ad26.ZEBOV and MVA-BN-Filo is under development by Janssen Vaccines and Prevention BV [12, 13]. The heterologous prime-boost vaccination regimens comprise one dose of each of these vaccine candidates with an intervening period of either 28 or 56 days; the second vaccine is administered to boost immune responses. In 2013, MVA-BN received market authorization in the EU and Canada as a smallpox vaccine [14]. Protection against Ebola virus disease using the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen is being evaluated in an extensive clinical trial program that includes healthy adults, adolescents, children ≥1 year of age and adults with human immunodeficiency virus (HIV) infection from different sub-Saharan countries.

We conducted a Phase 1 study in two urban/peri-urban, malaria-endemic areas of northwestern Tanzania and southwestern Uganda to assess the safety, tolerability, and immunogenicity of different Ad26.ZEBOV, MVA-BN-Filo heterologous prime-boost vaccination sequences, with a dosing interval of 28 or 56 days and a follow-up period of 1 year (VAC52150EBL1004; NCT02376400). This

study was designed to complement the VAC52150EBL1003 study, which was located in a high-altitude, urban setting with low incidence of malaria (described by Mutua *et al*. [15]).

Methods

Study population

Healthy adult volunteers (N=72), aged 18–50 years, were recruited from two malaria-endemic areas of East Africa: Mwanza (Tanzania) and Masaka (Uganda). Both sites are located near Lake Victoria at an altitude of approximately 1,100 meters. All study participants had to be healthy on the basis of physical examination, medical history, and the investigator's clinical judgment (see supplementary information for exclusion criteria).

Study design

The study design of this trial was identical to that described for the VAC52150EBL1003 trial by Mutua *et al.* [15]. (See supplementary information).

Study procedures

The protocol and procedures of this study followed exactly those of the VAC52150EBL1003 trial by Mutua *et al*. [15], including screening, individual randomization, and placebo-controlled vaccine administration.The primary objective was to assess the safety and tolerability of different Ad26.ZEBOV, MVA-BN-Filo heterologous prime-boost vaccination regimens. Secondary outcomes included Ebolavirus glycoprotein-specific humoral and cellular immune responses induced by the vaccine regimens.

Randomization and masking

Participants were randomized using a computer-generated block randomization schedule, and participants and study team members were blinded until 21 days post boost vaccination, as previously described [15].

Adverse event monitoring

The reporting of adverse events (AEs) was identical to that described for the VAC52150EBL1003 trial [15]. Briefly, solicited AEs were recorded in a diary by participants for 7 days following each vaccination and unsolicited AEs were collected at all visits until 21 days post boost. Solicited AEs were previously defined [15] (see supplementary information). Clinical AEs were graded according to the DMID scale [16], while laboratory toxicities were graded according to the FDA's Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [17]. AEs of special interest (AESIs) were recorded due to particular concerns with historic early generation MVA-based vaccines [18].

Immunogenicity measurements

Measurements of immunogenicity were identical to those previously described [15]. Immune responses were measured using serum samples taken before prime and boost immunizations, 7 days after prime and boost immunizations, and 21 days after boost immunizations. Participants who received vaccines with a 56-day interval had an additional blood draw 28 days after the prime immunization. Long-term follow-up samples were collected in all groups at Days 180, 240 and 360. IgG binding and neutralizing antibody responses were analyzed using enzyme-linked immunosorbent assay (ELISA) [13] and Pseudovirion neutralization assay (psVNA) [15], respectively. Exploratory objectives included evaluation of $CD4^+$/$CD8^+$ T cell responses using intracellular cytokine staining (ICS) flow cytometry [12, 19].

Data analysis and statistics

The primary analysis sets for safety and immunogenicity were as described previously [15]; the primary analysis set for safety (full analysis set) comprised all randomized participants who received

at least one dose of study vaccine. The primary analysis set for immunogenicity included all vaccinated participants with immunogenicity data at baseline and at least one measurement post-vaccination. All data were analyzed using descriptive statistics without formal hypothesis testing. Immunogenicity data are presented using similar methods to those of other Phase 1 studies of this prime-boost regimen [12, 15, 20]. A participant was defined as a responder for ELISA, VNA, or ICS at each time point if the test was negative at baseline and positive post-baseline, or if a positive baseline result was followed by at least a 3-fold increase, as described previously [15]. Given the small sample sizes in each vaccination group and minimal evidence available regarding statistical hypothesis testing, no formal statistical testing of safety data or immune responses was planned or performed.

Results

The study was conducted between February 18, 2015, and September 16, 2016. Seventy-two healthy adult African volunteers (25 from Tanzania and 47 from Uganda) were randomized into four groups of 18 volunteers; 15 were randomized to receive active vaccine and three to receive placebo (**Figure 1**). Active vaccine recipients were vaccinated with Ad26.ZEBOV or MVA-BN-Filo as prime and MVA-BN-Filo or Ad26.ZEBOV, respectively, as boost vaccination (n=30 each). For all participants, the inter-dose interval was either 28 or 56 days. Participants' baseline characteristics for placebo, MVA-BN-Filo and Ad26.ZEBOV (prime or boost) are shown in **Table 1**.

Safety and tolerability

Solicited local and systemic AEs were mostly mild-to-moderate and transient following MVA-BN-Filo (n=60) and Ad26.ZEBOV (n=59) administration. The most frequently reported solicited local AE was injection site pain for both vaccines and placebo (**Table 2**). One grade 3 solicited local AE was documented following Ad26.ZEBOV prime vaccination (injection site swelling). No grade 3 solicited local AEs were observed following administration of MVA-BN-Filo or placebo. The most frequently reported solicited systemic AEs were headache and fatigue for MVA-BN-Filo and Ad26.ZEBOV vaccines and also placebo (**Table 3**). Grade 3 solicited systemic AEs (headache in all cases) occurred in three participants: one MVA-BN-Filo boost recipient (considered by the investigator as doubtfully related to study vaccine as the participant had concurrent clinical malaria), one Ad26.ZEBOV boost recipient (considered by the investigator as doubtfully related to study vaccine and thought to be attributable to recurrent toothache), and one placebo recipient. The median duration of frequently reported solicited AEs ranged from 1–3 days following MVA-BN-Filo vaccination, and 1–2 days following Ad26.ZEBOV vaccination.

The frequency (% per dose) of all unsolicited AEs was 66.7%, 57.6% and 79.2% following vaccination with MVA-BN-Filo, Ad26.ZEBOV or placebo, respectively. The most frequent unsolicited AEs were

proteinuria (n=14 [n=9 post MVA-BN-Filo, 15%; n=2 post Ad26.ZEBOV, 3.4%; n=3 post placebo, 12.5%]), headache (n=11 [n=4 post MVA-BN-Filo, 6.7%; n=3 post Ad26.ZEBOV, 5.1%; n=4 post placebo, 16.7%]), decreased neutrophil count (n=10 [n=4 post MVA-BN-Filo, 6.7%; n=3 post Ad26.ZEBOV, 5.1%; n=3 post placebo, 12.5%]), and malaria (n=4 [n=1 post MVA-BN-Filo, 1.7%; n=3 post placebo, 12.5%]). These unsolicited AEs occurred more frequently in placebo recipients than in the active vaccine groups, with the exception of proteinuria, which was based on protein dipstick results from a mid-stream urine sample.

The majority of unsolicited AEs were grade 1 and 2 in severity, with grade 3 unsolicited AEs reported in 12 participants (20%) post MVA-BN-Filo, 9 (15.3%) post Ad26.ZEBOV and 4 (16.7%) post placebo. One grade 3 bradycardia event (heart rate <50 bpm) was reported as an AESI following MVA-BN-Filo prime vaccination because it was considered to be a cardiac sign or symptom. The bradycardia was considered to be probably related to study vaccine. The symptoms resolved within 1 hour without treatment. This participant did not receive a boost vaccination. All other grade 3 unsolicited AEs were related to laboratory abnormalities and, therefore, were reported as AEs regardless of clinical significance, as per protocol. These reported AEs were decreased neutrophil count (5 post MVA-BN-Filo; 3 post Ad26.ZEBOV; 3 post placebo), decreased hemoglobin (1 post MVA-BN-Filo; 2 post Ad26.ZEBOV), prolonged prothrombin time (1 post MVA-BN-Filo; 2 post Ad26.ZEBOV; 1 post placebo), proteinuria (3 post MVA-BN-Filo), hyperkalemia (1 post MVA-BN-Filo; 1 post Ad26.ZEBOV), and blood potassium increased (1 post MVA-BN-Filo). However, in all subjects reporting prolonged prothrombin time, the international normalized ratio was grade 0 or grade 1.

No differences were seen in AE patterns when comparing the post prime time periods with the post boost time periods, or between different vaccine sequences or intervals. One serious adverse event (SAE; grade 2 typhoid fever) was reported 36 days following Ad26.ZEBOV prime vaccination and was not considered related to study vaccine by the investigator. In addition to the grade 3 bradycardia, there was 1 other AESI: one Ad26.ZEBOV-primed individual had grade 1 hypertension on day 1 that

was considered to be probably related to study vaccine. However, as the event was reported after Ad26.ZEBOV vaccination and prior to receiving MVA-BN-Filo, it was no longer considered an AESI after unblinding of the study.

Immunogenicity

Binding antibody responses

High levels of binding antibodies to the EBOV glycoprotein were generated in response to all four vaccination regimens. In general, responses to placebo were low or not quantifiable.

For both dosing intervals with Ad26.ZEBOV prime vaccination, binding antibody responder rates (i.e. the proportion of participants showing a true response) increased to 93% at the time of MVA-BN-Filo boost (**Figure 2A**). At 21 days post boost (Day 50 or 78), 100% of participants in both Ad26.ZEBOV-primed groups demonstrated an antibody response, with geometric mean concentration (GMC) values rising to 5,256 and 10,613 ELISA units/mL (EU/mL) in the 28-day and 56-day interval groups, respectively (**Supplementary Table 1**).

With MVA-BN-Filo prime, binding antibody responder rates were low at the time of Ad26.ZEBOV boost (21% and 14% with 28-day and 56-day intervals, respectively). At 21 days post boost, responder rates rose to 100% for both dosing intervals (**Figure 2A**), with GMCs rising to 4,654 and 9,691 EU/mL in the 28-day and 56-day interval groups, respectively (**Supplementary Table 1**).

The magnitude of the binding antibody responses decreased towards Day 180 post prime but stabilized thereafter. Across all regimens, responses persisted in 100% of participants until Day 360 post prime, with GMCs ranging from 550–730 units/mL **(Figure 3A)**.

Virus neutralizing antibody (VNA) response

Neutralizing antibody titers were low following Ad26.ZEBOV prime vaccination but increased by 21 days post boost vaccination so that 93% and 100% of participants on the 28- and 56-day interval regimens, respectively, showed neutralizing antibody responses (**Figure 2B**). Geometric mean titer (GMT) values were elevated at 21 days post boost, at 1,001 IC_{50} titer and 3,042 IC_{50} titer in the 28-day and 56-day interval groups, respectively **(Figure 2B, Supplementary Table 1)**.

Neutralizing antibody titers were low following MVA-BN-Filo prime vaccination but increased by 21 days post boost vaccination so that 87% and 100% of participants on the 28- and 56-day interval regimens, respectively, showed neutralizing antibody responses **(Figure 2B)**. In the MVA-BN-Filo prime groups, GMTs at 21 days post boost were 439 IC_{50} titer and 2,297 IC_{50} titer for the 28- and 56-day intervals, respectively (**Supplementary Table 1**).

In all Ad26.ZEBOV and MVA-BN-Filo prime groups, GMTs declined to a stable level observed by Day 180, which was sustained until Day 360 (**Figure 3B**).

$CD8^+$ T cell responses

In the Ad26.ZEBOV prime groups, T cell responses were only observed following MVA-BN-Filo boosting. Responder rates peaked 21 days post boost at 58% for the 28-day interval group and remained elevated at Day 360 at 31% (**Figure 4A**). For the 56-day interval group, a peak responder rate of 50% was observed at 7 days post boost and was sustained until Day 180, after which it declined to 30% at Day 360. Median cytokine responses were highest at 7 and 21 days post boost for the 56-day and 28-day interval groups, respectively, at 0.07% and 0.09% **(Figure 4A).**

In the MVA-BN-Filo prime groups, a peak responder rate of 13% was observed 7 days post boost for the 28-day interval regimen. There were no responders at any time point in the 56-day interval group **(Figure 4A)**.

$CD4^+$ T cell responses

A peak $CD4^+$ T cell responder rate of 33% was observed at 21 days post boost in the Ad26.ZEBOV prime 28-day interval group. In the 56-day interval group, T cell responder rates peaked 7 days post boost at 50% (**Figures 4B**). In the Ad26.ZEBOV prime 28-day interval regimen, the median cytokine response was 0.09% at 21 days post boost. For the 56-day interval group, the highest median $CD4^+$

cytokine responses were reached at 21 days post boost with 0.14%. By Day 360, $CD4^+$ T cell responder rates in all Ad26.ZEBOV prime groups ranged from 0–20%.

While no responders were detected at any time point in the MVA-BN-Filo prime group with a 56-day interval, a peak responder rate of 25% was observed at 21 days post boost in the 28-day interval regimen. The responder rate was sustained until Day 180 (**Figure 4B**). The highest median cytokine response was also recorded at 21 days post boost, at 0.07%.

Discussion

This is the first clinical trial of the novel Ad26.ZEBOV, MVA-BN-Filo vaccination strategy that recruited participants from within a malaria-endemic region of Sub-Saharan Africa. The results of this study are similar to the findings from other Phase 1 studies of heterologous prime-boost regimens of Ad26.ZEBOV, MVA-BN-Filo and MVA-BN-Filo, Ad26.ZEBOV performed in Nairobi, Kenya [15] and the United Kingdom [12, 20], showing that these prime-boost vaccination regimens consistently demonstrate a favorable safety profile, eliciting few grade 3 AEs and no SAEs, in different regions. For both vaccine regimens, AE patterns were similar whether being administered as prime or boost vaccination, and with a 28 or 56 day dosing interval. Strong humoral immune responses were observed, reaching binding and neutralizing antibody responder rates of 100% and 87–100%, respectively, at 21 days post boost, regardless of vaccine interval and sequence. Although the magnitude of responses declined over time, the responder rate remained high at 1 year post prime (100% of volunteers showed binding antibodies and 53–87% showed neutralizing antibodies). Post prime, T cell responses were low or not quantifiable, and post boost the highest responses were observed for participants receiving the Ad26.ZEBOV-primed regimen with either the 28-day or 56-day prime-boost interval. This finding is consistent with data reported previously [12]. Extending the dosing interval from 28 days to 56 days led to an increase in humoral responses. Efficacy data with the heterologous prime-boost Ad26.ZEBOV, MVA-BN-Filo or MVA-BN-Filo, Ad26.ZEBOV regimens are not yet available in humans; however, non-human primate data have shown a strong correlation between binding antibody responses and survival after challenge with EBOV [21].

Both this study and that of Mutua *et al.* [15] are part of the clinical development program for Ad26.ZEBOV, MVA-BN-Filo prime-boost vaccination, designed to ascertain the potential of this vaccination strategy to play a role in the prevention and/or containment of future Zaire Ebolavirus outbreaks. The need for effective control measures was highlighted by the recent outbreaks in the Democratic Republic of the Congo [8–10]. The re-emergence of Ebola virus is unpredictable,

suggesting a need either for population-wide protection, healthcare worker protection or alternative effective control measures that can be implemented rapidly.

With our findings and those of Mutua *et al*. [15], there are now data from multiple studies demonstrating the safety and tolerability of these vaccines in different African populations from geographical areas representative of the region. In addition, data have also been captured from the United Kingdom in a population geographically and ethnically distinct from those experiencing the Ebola virus outbreak [12, 20]. In our study, similar to the findings from the UK, injection site pain was the most frequently reported solicited local AE, and the most common solicited systemic AEs were headache, fatigue and myalgia, all of which occurred with mild-to-moderate severity and were of short duration [12]. In Uganda and Tanzania, fever, a common symptom of Ebola [22], was not a frequent solicited systemic AE, and no vaccine-related SAEs were reported.

In our study, conducted in malaria-endemic communities on Lake Victoria [23, 24], four out of 72 (5.5%) participants developed clinical malaria, with three of these participants in the placebo arm. The total proportion of participants reporting malaria was the same as in the VAC52150EBL1003 study [15] conducted in Nairobi, which is not endemic for malaria and where participants were therefore considered to be at a low risk of the infection unless they travelled out of the city [25]. Our study showed a similar pattern to a previous study from West Africa with the ChAd3-EBO-Z Ebola vaccine, as both demonstrated an unexpected finding of lower incidence of malaria in participants receiving the active vaccine compared to participants receiving placebo [26]. However, the small sample size in our study makes it difficult to assess any association between the vaccines and a possible lower risk of malaria.

Several other candidate prophylactic Ebola vaccines have also been tested in clinical trials in Africa. Over 4000 individuals received the rVSV-ZEBOV vaccine in a Phase 3 study of ring vaccination; vaccine efficacy was estimated to be 100% (95% CI 68.9–100) and only three SAEs occurred that

were judged at least possibly related to vaccination (1 febrile reaction, 1 anaphylaxis and 1 influenza-like illness; all resolved without further sequelae) [27]. Consequently, this vaccine was used in the 2018 outbreaks in the Democratic Republic of the Congo [28, 29]. Comparisons of immune responses in different studies need to be made with caution, as they may be confounded by differences in population characteristics, dosing regimens, or the use of different assays to measure antibody responses. However, when using the same ELISA protocol, the Ad26.ZEBOV, MVA-BN-Filo heterologous regimens in this study and that of Mutua *et al.* [15], especially the 56-day interval regimens, induced higher EBOV-specific glycoprotein IgG concentrations post boost than single-dose rVSV-ZEBOV [30]. Another candidate vaccine, ChAd3-EBO-Z, is an adenovirus-based vaccine expressing Zaire Ebolavirus glycoprotein and can be boosted by MVA-BN-Filo [31]. The persistence of binding antibody responses observed in the current study up to one year post Ad26.ZEBOV, MVA-BN-Filo vaccination has also been observed for both the rVSV-ZEBOV vaccine and the ChAd3-EBO-Z vaccine in study participants in Liberia [26].

Our study provides support to the further development of the Ad26.ZEBOV, MVA-BN-Filo heterologous prime-boost vaccination strategy. Further clinical studies are currently underway to evaluate Ad26.ZEBOV prime and MVA-BN-Filo boost vaccination, with dosing intervals of 28, 56, and 84 days (NCT02564523 and NCT02509494). Different populations are being included in these studies (e.g. children and individuals with HIV; NCT02564523 and NCT02509494). Ad26.ZEBOV, MVA-BN-Filo clinical trials are also investigating short duration regimens with intervals between prime and boosting of 7 or 14 days (NCT02325050 and NCT02598388). Prime-boost regimens with longer dosing intervals, e.g. a 56-day interval, tend to elicit higher antibody responses, as demonstrated in this study, and therefore they may be more suitable for long-term protection strategies. Conversely, short intervals between prime and boost vaccinations may enable reactive use and early onset of immunity in the context of an outbreak.

In two placebo recipients (one from Uganda; one from Tanzania), receiving 56-day interval booster vaccination depending on timepoint, binding antibodies were detected. A potential explanation may be previous asymptomatic infection through exposure of populations to the virus, particularly in Uganda where Ebola virus outbreaks have previously occurred, or the circulation of unknown but closely related virus strains in these settings. There have been approximately 25 outbreaks of Ebola virus in Africa alone since 1976, in addition to the virus being transmitted to non-endemic countries [5], and this demonstrates the need for continued development of vaccines against Ebola, even after the end of the last epidemic.

Although the relatively small number of participants might be considered to be a limitation of our study, the similarity in findings of our study and those of Mutua *et al.* [15], conducted independently in different countries of East Africa, strengthen the conclusions that can be made. Strengths of the study include the exploration of multiple vaccination regimens, and the 12-month follow-up period enabling the durability of immune responses to be assessed.

In conclusion, this study has demonstrated that heterologous Ad26.ZEBOV, MVA-BN-Filo prime-boost vaccination against Ebola is well tolerated and immunogenic in healthy African adult volunteers, regardless of whether the dosing interval is 28 or 56 days. Ad26.ZEBOV priming induced more robust initial antibody and T cell responses than MVA-BN-Filo priming, and immune responses were shown to persist for at least 360 days. The results of later Phase trials of Ad26.ZEBOV, MVA-BN-Filo prime-boost vaccination are to be reported.

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Author contributions

All authors had full access to the data. The corresponding author had final responsibility for the decision to submit for publication.

Conflict of interest

Viki Bockstal, Georgi Shukarev, Dickson Anumendem, Kerstin Luhn, Cynthia Robinson and Macaya Douoguih are all full-time employees of Janssen Pharmaceuticals Inc. or its affiliates.

All other authors: No potential conflicts of interest.

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Table 1: Baseline characteristics of study participants

	Boost at Day 28			Boost at Day 56		
	MVA, Ad26	**Ad26, MVA**	**Placebo**	**MVA, Ad26**	**Ad26, MVA**	**Placebo**
	n=15	**n=15**	**n=6**	**n=15**	**n=15**	**n=6**
Sex, n (%)						
Female	1 (6.7)	3 (20.0)	2 (33.3)	3 (20.0)	5 (33.3)	1 (16.7)
Male	14 (93.3)	12 (80.0)	4 (66.7)	12 (80.0)	10 (66.7)	5 (83.3)
Median age (range), years	25 (20–41)	24 (20–37)	27 (24–49)	27 (18–38)	25 (19–42)	23 (20–43)
Median body mass index (range), kg/m^2	21.1 (18.2–23.6)	23.2 (16.1–35.4)	20.4 (18.4–28.5)	22.2 (15.9–30.7)	21.8 (18.6–33.8)	21.0 (17.5–26.9)

Table 2: Frequency (% per dose) of solicited local adverse events following priming and boosting with standard doses of MVA-BN-Filo and Ad26.ZEBOV (pooled prime and boost data from all four vaccination regimens)

Solicited local AEs		MVA n=60	Ad26.ZEBOV n=59	Placebo n=24
Any solicited local AE, n (%)	Grade 1	30 (50)	24 (41)	12 (50)
	Grade 2	14 (23)	15 (25)	0 (0)
	Grade 3	0 (0)	1 (2)	0 (0)
	Total	44 (73)	40 (68)	12 (50)
Injection site pain, n (%)*	Grade 1	31 (52)	26 (44)	10 (42)
	Grade 2	11 (18)	13 (22)	0 (0)
	Total	42 (70)	39 (66)	10 (42)
Injection site warmth, n (%)*	Grade 1	15 (25)	13 (22)	6 (25)
	Grade 2	6 (10)	2 (3)	0 (0)
	Total	21 (35)	15 (25)	6 (25)
Injection site pruritus, n (%)*	Grade 1	12 (20)	8 (14)	6 (25)
	Grade 2	2 (3)	1 (2)	0 (0)
	Total	14 (23)	9 (15)	6 (25)
Injection site swelling, n (%)¶	Grade 3	0 (0)	1 (2)	0 (0)
	Total	0 (0)	1 (2)	0 (0)

*No Grade 3 AEs reported; ¶No Grade 1 or 2 AEs reported

Table 3: Frequency (% per dose) of solicited systemic adverse events (AEs) following priming and boosting with standard doses of MVA-BN-Filo and Ad26.ZEBOV (pooled prime and boost data from all 4 vaccination regimens)

Solicited systemic AEs		**MVA**	**Ad26**	**Placebo**
		n=60	**n=59**	**n=24**
Any solicited systemic AE, n (%)	Grade 1	27 (45)	18 (31)	10 (42)
	Grade 2	18 (30)	25 (42)	4 (17)
	Grade 3	1 (2)	1 (2)	1 (4)
	Total	46 (77)	44 (75)	15 (63)
Headache, n (%)				
	Grade 1	23 (38)	19 (32)	8 (33)
	Grade 2	10 (17)	13 (22)	2 (8)
	Grade 3	1 (2)	1 (2)	1 (4)
	Total	34 (57)	33 (56)	11 (46)

Fatigue, n (%)*				
	Grade 1	21 (35)	18 (31)	5 (21)
	Grade 2	9 (15)	15 (25)	2 (8)
	Total	30 (50)	33 (56)	7 (29)
Myalgia, n (%)*	Grade 1	18 (30)	12 (20)	4 (17)
	Grade 2	10 (17)	8 (14)	1 (4)
	Total	28 (47)	20 (34)	5 (21)
	Total	28 (47)	20 (34)	5 (21)
Arthralgia, n (%)*	Grade 1	14 (23)	9 (15)	3 (13)
	Grade 2	4 (7)	6 (10)	0 (0)
	Total	18 (30)	15 (25)	3 (13)
Nausea, n (%)*	Grade 1	12 (20)	12 (20)	1 (4)
	Grade 2	3 (5)	5 (9)	1 (4)

	Total	15 (25)	17 (29)	2 (8)
Chills, n (%)*	Grade 1	8 (13)	11 (19)	1 (4)
	Grade 2	3 (5)	1 (2)	0 (0)
	Total	11 (18)	12 (20)	1 (4)
Pruritus (generalized), n (%)*	Grade 1	5 (8)	2 (3)	2 (8)
	Grade 2	2 (3)	3 (5)	1 (4)
	Total	7 (12)	5 (8)	3 (13)
Rash, n (%)*	Grade 1	5 (8)	3 (5)	1 (4)
	Grade 2	1 (2)	0 (0)	0 (0)
	Total	6 (10)	3 (5)	1 (4)
Pyrexia, n (%)*	Grade 1	1 (2)	2 (3)	0 (0)
	Grade 2	0 (0)	2 (3)	0 (0)
	Total	1 (2)	4 (7)	0 (0)

Vomiting, n (%)[†]	Grade 1	0 (0)	3 (5)	0 (0)
	Total	0 (0)	3 (5)	0 (0)

*No Grade 3 AEs reported; [†]No Grade 2 or 3 AEs reported

Figure 1: Subject disposition

*One subject did not receive Ad26.ZEBOV boost vaccination, because he met a protocol-specific criterion for contraindication to boost. This subject remained in the study.

Figure 2: Anti-EBOV glycoprotein IgG (ELISA) binding antibody responses **(A)** and virus neutralizing antibody (VNA) **(B)** following priming with MVA-BN-Filo or Ad26.ZEBOV and heterologous boosting with Ad26.ZEBOV or MVA-BN-Filo on Day 29 or Day 57, up to 21 days post boost (day 50 or 78). Data shown are geometric mean values (concentrations [GMC] and titers [GMT] for ELISA and VNA, respectively); error bars represent 95% confidence intervals. NA, not applicable

A: Binding antibody response

B: Virus neutralizing antibody response

Figure 3: Durability of anti-EBOV glycoprotein IgG binding **(A)** and neutralizing **(B)** antibody responses following priming with MVA-BN-Filo or Ad26.ZEBOV and heterologous boosting with Ad26.ZEBOV or MVA-BN-Filo on Day 29 or Day 57. Data shown are geometric mean values (concentrations [GMC] and titers [GMT] for ELISA and VNA, respectively); error bars represent 95% confidence intervals

A: Anti-EBOV GP Binding Antibody Response

B. Anti-EBOV GP Neutralizing Antibody Response

Figure 4: Median $CD8^+$ **(A)**, and $CD4^+$ T cell responses **(B)** following priming with MVA-BN-Filo or Ad26.ZEBOV and heterologous boosting with Ad26.ZEBOV or MVA-BN-Filo on Day 29 or Day 57. NA, not applicable

A: $CD8^+$ T cell response

B: $CD4^+$ T cell response

Days

Figure 1

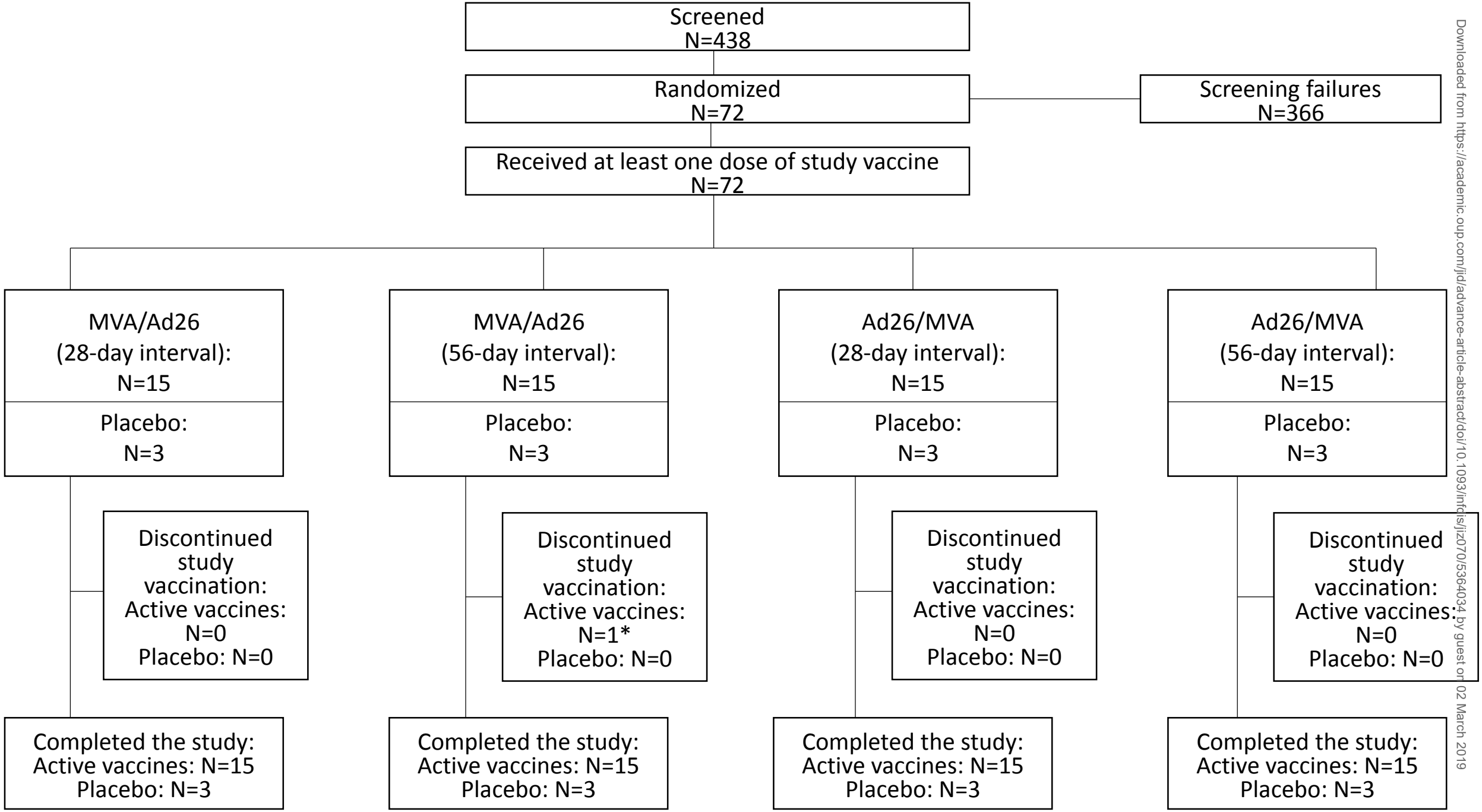

Figure 2A

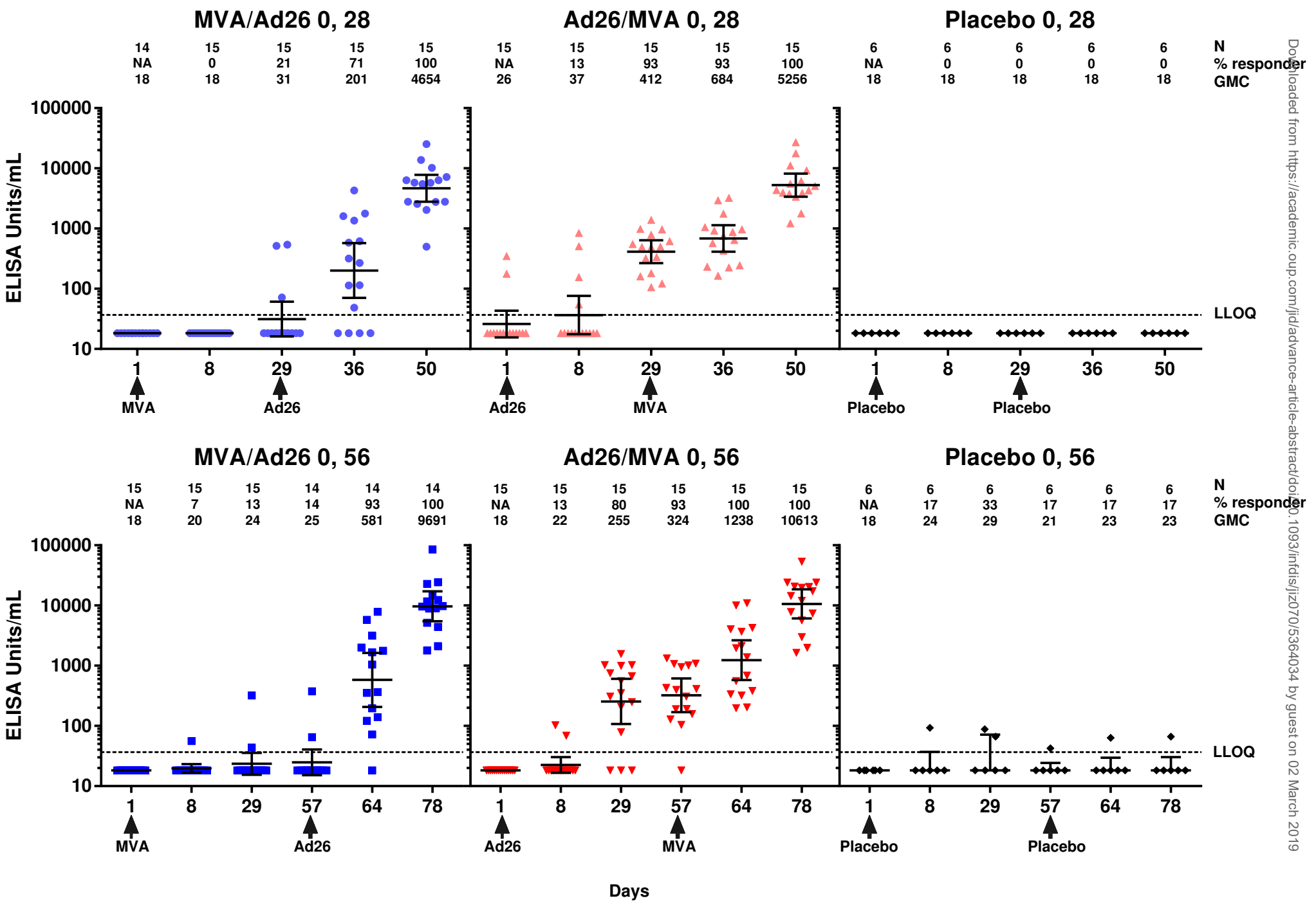

Figure 2B

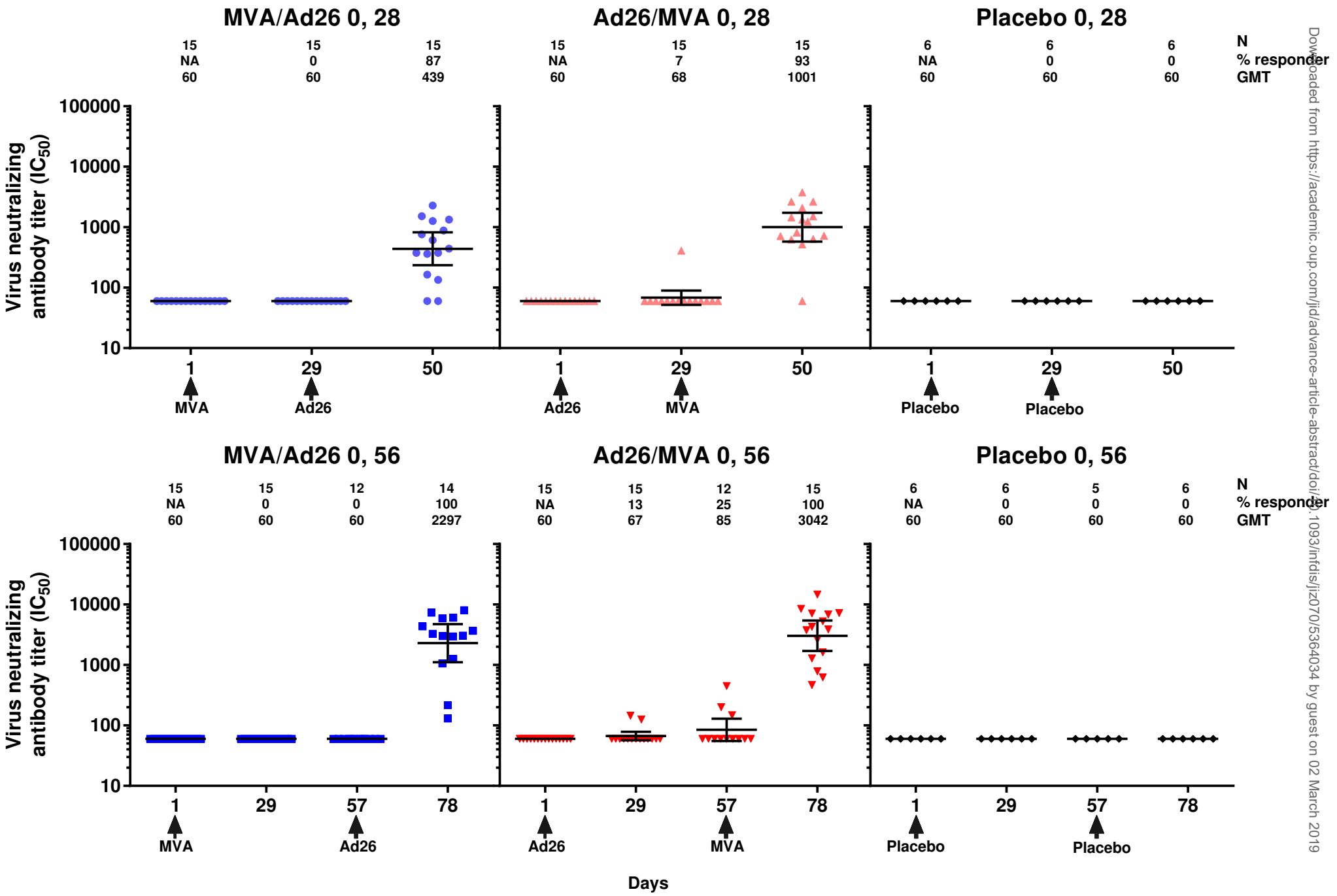

Figure 3A

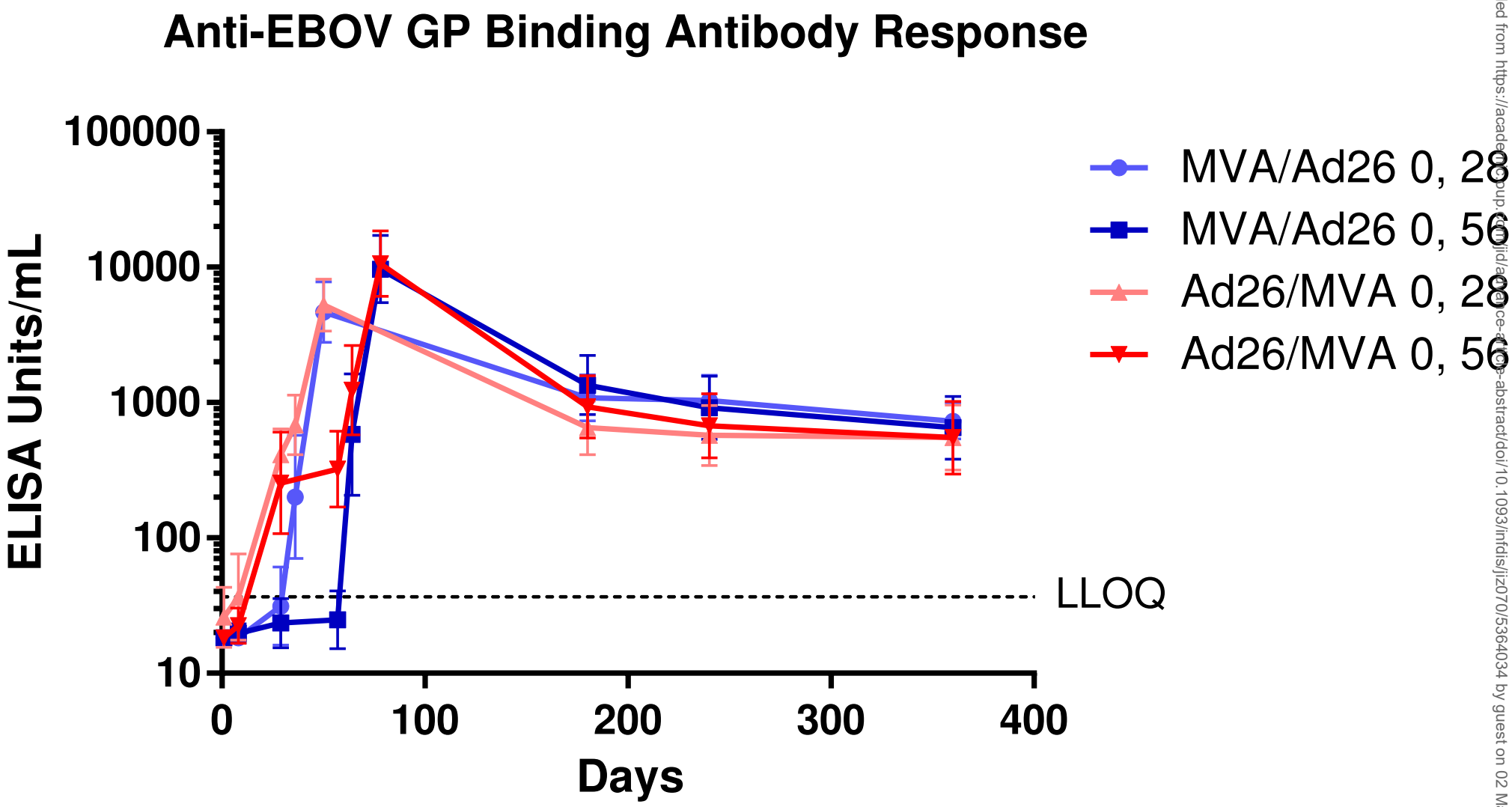

Figure 3B

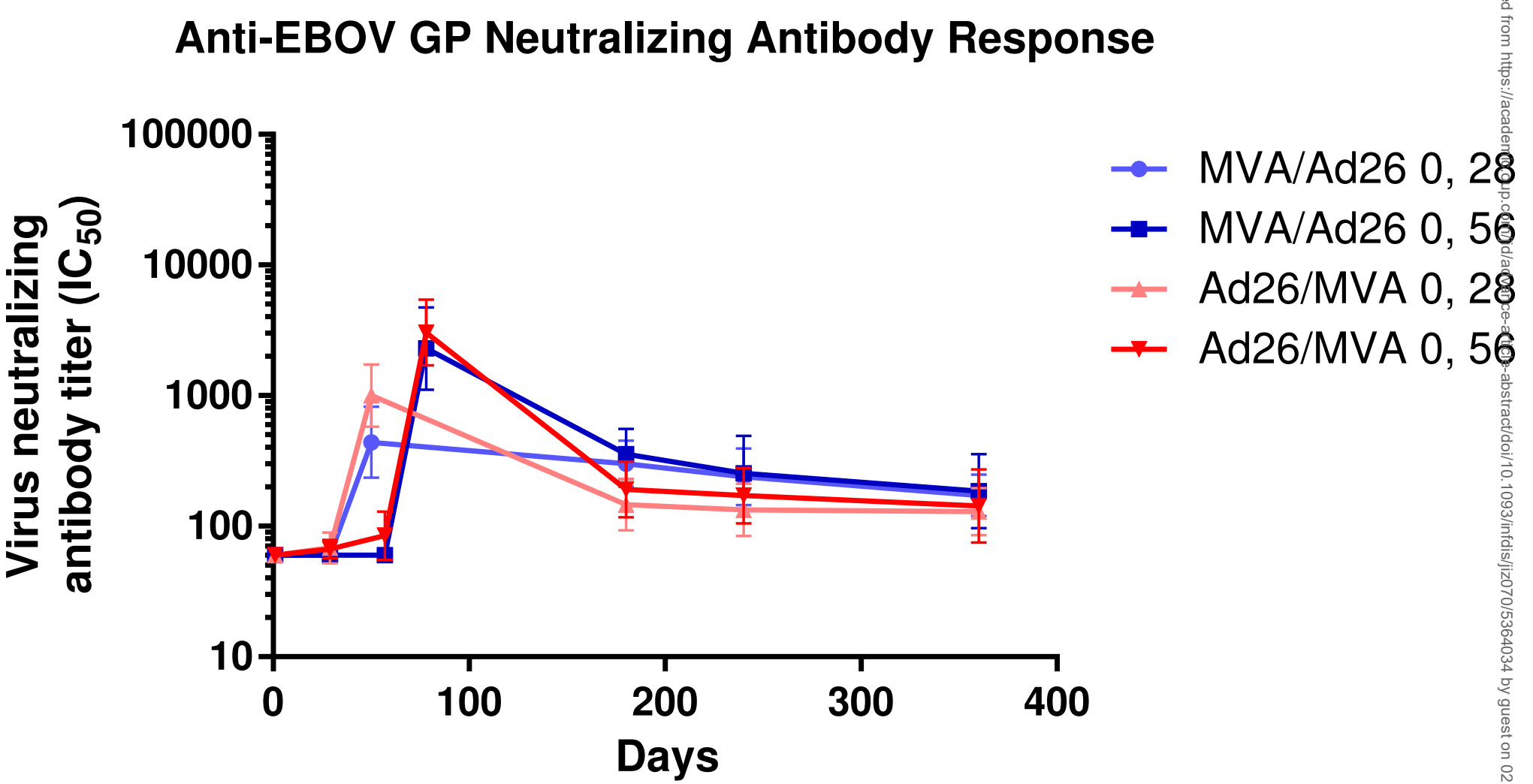

Figure 4A

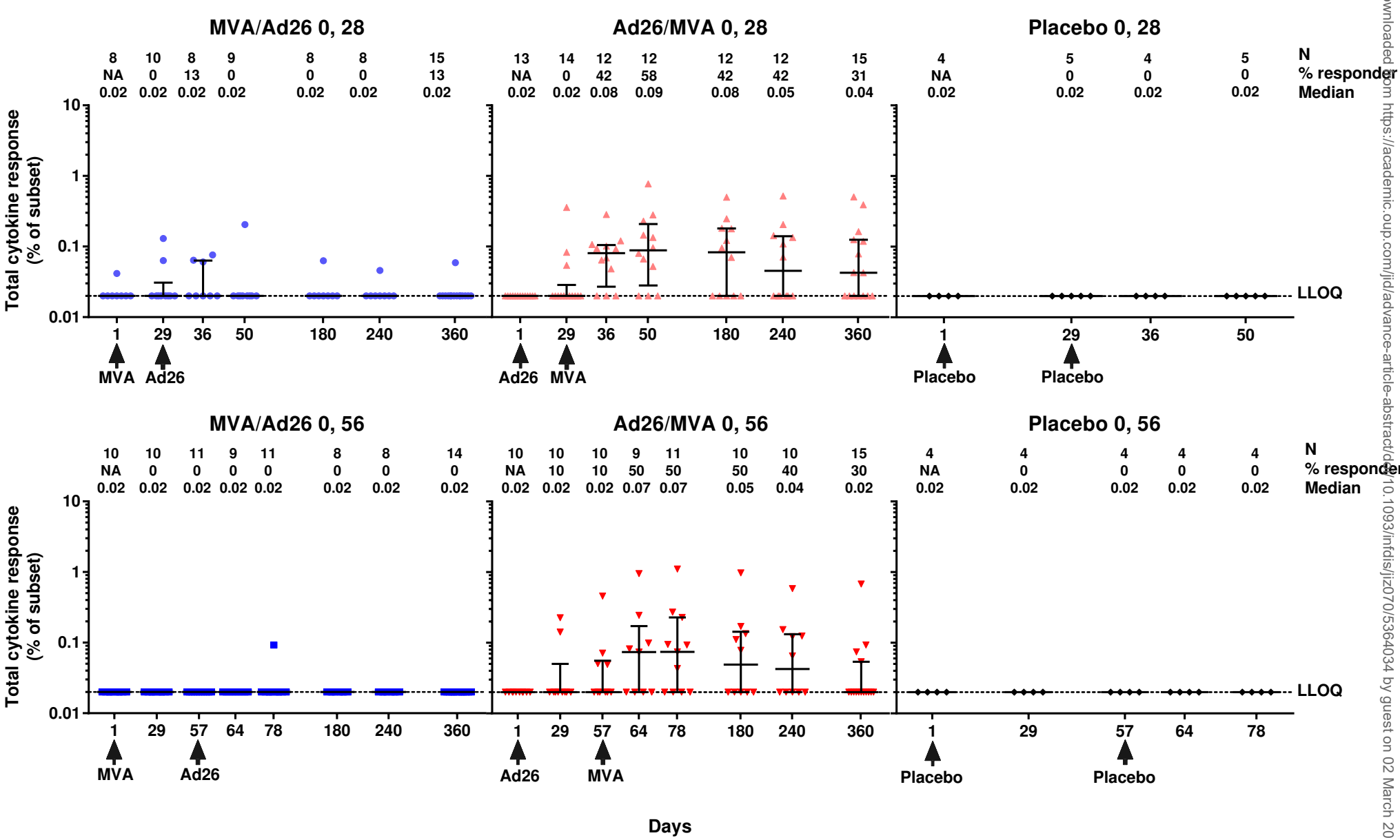

Figure 4B