

Immune responses to Novel Adenovirus Type 26 and Modified Vaccinia Virus

Ankara-Vectored Ebola Vaccines at 1 year.

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20 **Introduction**

21 The Ebola virus vaccine strategies evaluated by the WHO in response to the recent outbreak
22 in West Africa included a heterologous prime/boost schedule of the adenovirus type 26
23 vector vaccine encoding Ebola virus glycoprotein (Ad26.ZEBOV) and the modified vaccinia
24 virus Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg
25 virus, and Tai Forest virus nucleoprotein (MVA-BN-Filo). This schedule has been shown to
26 induce immune responses that persist for 8-months post priming immunization, with 100%
27 of vaccine recipients retaining Ebola virus glycoprotein-specific antibodies.¹

28 A vaccine that provides durable immune responses is important in maintaining sustained
29 protection against disease, both during outbreaks (given the prolonged duration of the
30 2014-2016 Ebola outbreak) and outside of an outbreak for at-risk populations. At-risk
31 populations include health care workers in risk areas, international rapid response team
32 members, aid workers, individuals in areas experiencing low-grade endemic disease or
33 ongoing disease risk^{2,3}, and contacts of Ebola survivors, given evidence of prolonged
34 shedding of the virus from body fluids for up to 9-months following infection⁴ with the
35 potential for transmission.⁵

36 We report the 1-year data for the above study of the Ad26.ZEBOV/MVA-BN-Filo vaccines,
37 the longest duration follow-up for any Ebola vaccine candidate to our knowledge.

38 **Methods**

39 The single-center, randomized, placebo-controlled, observer-blind, phase 1 trial was
40 performed in Oxford, United Kingdom, enrolling 87 healthy 18 to 50-year olds from
41 December 2014 following written informed consent; 12-month follow-up was completed

March 2016 (ethical approval 14/SC/1408). 72 participants were randomized to 4 groups, each with 18 participants (3 receiving placebo and 15 active vaccine). Individuals in the vaccine groups received either Ad26.ZEBOV (5×10^{10} viral particles) or MVA-BN-Filo (1×10^8 median tissue culture infective dose) first, followed by boosting with the alternate vaccine 28 or 56-days later. An open-label fifth group consisted of an additional 15 participants vaccinated with Ad26.ZEBOV followed by MVA-BN-Filo 14-days later.

The primary outcome was adverse event collection. Secondary outcomes were the magnitude of humoral and cellular immune responses assessed by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISpot) and the percentage of vaccine responders (see Table 1 for definition). The number of CD4+ and CD8+ T cells and their cytokine expression patterns were assessed by intracellular cytokine staining (ICS), as exploratory outcomes. Data analysis was descriptive (using SAS version 9.2) without formal statistical testing. Collection of day 360 data was a preplanned secondary analysis for vaccine recipients only; further details are available in the protocol (see supplementary material of the original publication).¹

Results

64 of 75 active vaccine recipients attended follow-up at day 360 (median age 39 years, 66% female). 11 participants withdrew (1 to 3 per group) and missing data were not imputed. No serious adverse events were recorded between day 240 and day 360.

One-hundred percent of these individuals maintained Ebola virus-specific IgG responses at day 360 (Figure 1; Table 1). Vaccine-induced T cell responses persisted in 60-83% of participants receiving Ad26.ZEBOV prime followed by MVA-BN-Filo boost, compared with 69-100% of those receiving the reverse regimen (Table 1). Persistence of the CD8+ and CD4+

65 responses is shown in Table 1.

66 **Discussion**

67 Immunity after heterologous prime/boost Ad26.ZEBOV/MVA-BN-Filo immunization
68 persisted at 1-year. Although no correlate of protection has yet been established, Ebola
69 virus glycoprotein-specific antibodies appear to play an important role in immunity.⁶ A
70 strategy of pre-emptive use of an AD26.ZEBOV/MVA-BN-Filo immunization schedule in ‘at
71 risk’ populations (where durability of immune response is likely to be of primary
72 importance) may offer advantages over reactive use of single-dose vaccine regimens^{2,3,7}. It
73 is important to note the limitation that this study was conducted in a European population;
74 immune responses may differ in a sub-Saharan African population and accordingly these
75 vaccine candidates are being further assessed in phase II/III studies in this region. Additional
76 research is also warranted to explore the persistence of immunity beyond 1 year following
77 immunization and response to booster doses of vaccine.

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79 ARTICLE INFORMATION

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84 *Statistical analysis:* Voysey

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Figure 1: Ebola glycoprotein–specific antibody responses for vaccine recipients (ELISA units/ml).

Data shown are geometric mean and 95% confidence interval.

Ad26: adenovirus-type 26 vector vaccine encoding Ebola glycoprotein. MVA: modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus and Tai Forest virus nucleoprotein. Day 1 is baseline, the day of first vaccination. Dotted lines represent booster vaccinations. For numbers included at each time point, see table 1.

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Table 1. Ebola Glycoprotein-Specific Antibody Responses detected by ELISA; Ebola Glycoprotein-Specific T-Cell Responses as assessed by Interferon-γ ELISpot; Ebola-specific CD4+ and CD8+ T cells as assessed by intracellular cytokine staining.

	Prime on Day 1, Boost on Day 29		Prime on Day 1, Boost on Day 57		Prime on Day 1, Boost on Day 15
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	Ad26.ZEBOV, Then MVA-BN-Filo
ELISA					
Day 240					
No of participants	14	15	11	13	11
Geometric mean concentration (95% CI) ELISA units/mL	3740 (2511.4; 5569.3)	2443 (1344.1; 4440.4)	3038 (1958.3; 4713.3)	2241 (1555.5; 3228.1)	1541 (860.2; 2760.8)
Responder, No (%) ^a	14 (100%)	15 (100%)	14 (100%)	13 (100%)	11(100%)
95% CI	76.8-100	78.2-100	76.8-100	75.3-100	71.5-100
Day 360					
No of participants (60)	14	13	12	12	9
Geometric mean concentration (95% CI) ELISA units/mL	3941 (2460.1; 6315)	1719 (830.4; 3557.4)	2540 (1589.9; 4058.5)	1738 (1206.8; 2504.2)	1468 (717.7; 3003.6)
Responder, No (%) ^a	14 (100%)	13 (100%)	12 (100%)	12 (100%)	9 (100%)
95% CI	76.8-100	75.3-100	73.5-100	73.5-100	66.4-100
ELISpot – pooled					
Day 240					
No of participants	14	15	14	13	11
Median (IQR), SFUs/million cells	485 (95-810)	266.6 (110-395)	419.2 (281.7-461.7)	216.7 (100-585)	278.3 (63.3-510)
Responder, No (%) ^a	11 (78.6%)	12 (80%)	14 (100%)	10 (76.9%)	9 (81.8%)
95% CI	49.2-95.3	51.9-95.7	76.8-100	46.2-95.0	48.2-97.7
Day 360					
No of participants (60)	13	13	12	12	10
Median (IQR), SFUs/million cells	236.7 (105-716.7)	163.3 (73.3-295)	310.8 (175-379.2)	285.8 (101.7-399.2)	317.5 (<LLOQ-526.7)
Responder, No (%) ^a	9 (69.2%)	8 (61.5%)	12 (100%)	10 (83.3%)	6 (60%)

95% CI	38.6-90.9	31.6-86.1	73.5-100	51.6-97.9	26.2-87.8
CD4+ (ICS)					
Day 240					
No of participants	14	15	14	13	11
Median (IQR), SFUs/million cells	0.09 (<LLOQ-0.15)	0.04 (<LLOQ-0.10)	<LLOQ (<LLOQ-0.11)	0.06 (<LLOQ-0.08)	<LLOQ (<LLOQ-0.06)
Responder, No (%) ^a	7 (50%)	5 (33.3%)	4 (30.8%)	2 (15.4%)	1 (9.1%)
95% CI	23-77	11.8-61.6	9.1-61.4	1.9-45.5	0.23-41.3
Day 360					
No of participants (59)	13	13	11	12	10
Median (IQR), SFUs/million cells	0.08 (0.05-0.14)	0.05 (<LLOQ-0.06)	0.04 (<LLOQ-0.13)	0.05 (<LLOQ-0.12)	<LLOQ (<LLOQ-0.06)
Responder, No (%) ^a	4 (30.8%)	1 (7.7%)	4 (40%)	3 (25%)	1 (10%)
95% CI	9.1-61.4	0.19-36	12.2-73.8	5.5-57.2	0.25-44.5
CD8+ (ICS)					
Day 240					
No of participants	14	15	14	13	11
Median (IQR), SFUs/million cells	0.127 (<LLOQ-0.91)	0.176 (<LLOQ-0.72)	0.304 (0.23-0.39)	0.212 (0.08-0.39)	0.074 (0.05-0.39)
Responder, No (%) ^a	10 (71.4%)	9 (60%)	13 (92.9%)	11 (84.6%)	6 (54.5%)
95% CI	41.9-91.6	32.3-83.7	66.1-99.8	54.6-98.1	23.4-83.3
Day 360					
No of participants (59)	13	13	11	12	10
Median (IQR), SFUs/million cells	0.155 (<LLOQ-0.57)	0.135 (<LLOQ-0.17)	0.246 (0.20-0.60)	0.185 (0.09-0.44)	0.09 (<LLOQ-0.40)
Responder, No (%) ^a	8 (61.5%)	8 (61.5%)	11 (100%)	11 (91.7%)	6 (60%)
95% CI	31.6-86.1	31.6-86.1	31.6-86.1	61.5-99.8	26.2-87.8

Abbreviations: Ad26.ZEBOV, adenovirus type 26 vector vaccine encoding Ebola glycoprotein; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot on frozen peripheral blood mononuclear cells shown as number of spot-forming units (SFUs) per million cells; ICS intracellular staining; CI, exact Clopper-Pearson confidence interval; IQR, interquartile range; No., number of participants with data and for responders, number of participants with data at baseline and at post baseline time point.

^a Responders for ELISA, ELISpot or ICS were those participants whose results were negative at baseline and positive after baseline or positive at baseline with at least 3-fold increase from baseline.

