Letters

RESEARCH LETTER

Immune Responses to Novel Adenovirus Type 26 and Modified Vaccinia Virus Ankara–Vectored Ebola Vaccines at 1 Year

The Ebola virus vaccine strategies evaluated by the World Health Organization in response to the 2014-2016 outbreak in West Africa included a heterologous primary and booster vaccination schedule of the adenovirus type 26 vector vaccine encoding Ebola virus glycoprotein (Ad26.ZEBOV) and the modified vaccinia virus Ankara vector vaccine, encoding glycoproteins from Ebola, Sudan, Marburg, and Tai Forest viruses nucleoprotein (MVA-BN-Filo). This schedule has been shown to induce immune responses that persist for 8 months after primary immunization, with 100% of vaccine recipients retaining Ebola virus glycoprotein-specific antibodies.[1]

A vaccine that provides durable immune responses is important in maintaining sustained protection against disease, both during outbreaks and outside of an outbreak for at-risk populations, such as health care and aid workers in risk areas, individuals in areas experiencing low-grade endemic disease,[2] and contacts of Ebola survivors, given evidence of prolonged shedding of the virus from body fluids with the potential for transmission.[3,4]

We report the 1-year data for the study of the Ad26.ZEBOV and MVA-BN-Filo vaccines,[1] the longest duration follow-up for any heterologous primary and booster Ebola vaccine schedule to our knowledge.

Methods | The single-center, randomized, placebo-controlled, observer-blind, phase 1 trial received approval from the National Research Ethics Service. Participants provided written

Table. Ebola Antibody and T-Cell Responses Detected by ELISA, Interferon-γ ELISpot, and Intracellular Cytokine Staining at 1-Year Follow-up (Day 360)

	Prime on Day 1, Boost on Day 29		Prime on Day 1, Boost on Day 57		Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	
Ebola Glycoprotein-Specific Antibody Responses Assessed by ELISA					
Day 240					
No. of participants	14	15	11	13	11
Geometric mean concentration (95% CI), ELISA U/mL	3740 (2511-5569)	2443 (1344-4440)	3038 (1958-4713)	2241 (1556-3228)	1541 (860-2761)
Responder, No. (%) [95% CI][a]	14 (100) [77-100]	15 (100) [78-100]	14 (100) [77-100]	13 (100) [75-100]	11(100) [72-100]
Day 360					
No. of participants (n = 60)	14	13	12	12	9
Geometric mean concentration (95% CI), ELISA U/mL	3941 (2460-6315)	1719 (830-3557)	2540 (1590-4059)	1738 (1207-2504)	1468 (718-3004)
Responder, No. (%) [95% CI][a]	14 (100) [77-100]	13 (100) [75-100]	12 (100) [74-100]	12 (100) [74-100]	9 (100) [66-100]
Ebola Glycoprotein-Specific T-Cell Responses Assessed by Interferon-γ ELISpot (Pooled)[b]					
Day 240					
No. of participants	14	15	14	13	11
Median (IQR), SFUs/million cells	485 (95-810)	267 (110-395)	419 (282-462)	217 (100-585)	278 (63-510)
Responder, No. (%) [95% CI][a]	11 (79) [49-95]	12 (80) [52-96]	14 (100) [77-100]	10 (77) [46-95]	9 (82) [48-98]
Day 360					
No. of participants (n = 60)	13	13	12	12	10
Median (IQR), SFUs/million cells	237 (105-717)	163 (73-295)	311 (175-379)	286 (102-399)	318 (<LLOQ-527)
Responder, No. (%) [95% CI][a]	9 (69) [39-91]	8 (62) [32-86]	12 (100) [74-100]	10 (83) [52-98]	6 (60) [26-88]
Ebola-Specific CD4+ T Cells Assessed by Intracellular Cytokine Staining					
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. (%) [95% CI][a]	7 (50) [23-77]	5 (33) [12-62]	4 (31) [9-61]	2 (15) [2-45]	1 (9) [0-41]
Day 360					
No. of participants (n = 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. (%) [95% CI][a]	4 (31) [9-61]	1 (8) [0-36]	4 (40) [12-74]	3 (25) [5-57]	1 (10) [0-45]

(continued)

Table. Ebola Antibody and T-Cell Responses Detected by ELISA, Interferon-γ ELISpot, and Intracellular Cytokine Staining at 1-Year Follow-up (Day 360) (continued)

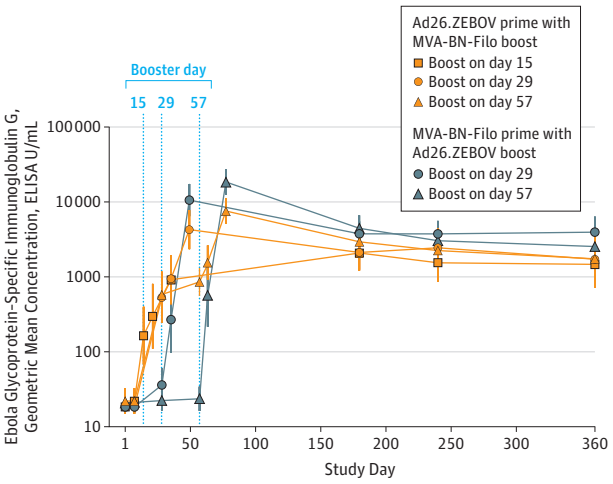
	Prime on Day 1, Boost on Day 29		Prime on Day 1, Boost on Day 57		Prime on Day 1, Boost on Day 15: Ad26.ZEBOV, Then MVA-BN-Filo
	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	MVA-BN-Filo, Then Ad26.ZEBOV	Ad26.ZEBOV, Then MVA-BN-Filo	
Ebola-Specific CD8+ T Cells Assessed by Intracellular Cytokine Staining					
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. (%) [95% CI][a]	10 (71) [42-92]	9 (60) [32-84]	13 (93) [66-100]	11 (85) [55-98]	6 (55) [23-83]
Day 360					
No. of participants (n = 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)	0.090 (<LLOQ-0.40)
Responder, No. (%) [95% CI][a]	8 (62) [32-86]	8 (62) [32-86]	11 (100) [32-86]	11 (92) [62-100]	6 (60) [26-88]

Abbreviations: Ad26.ZEBOV, adenovirus type 26 vector vaccine encoding Ebola glycoprotein; CI, exact Clopper-Pearson confidence interval; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; IQR, interquartile range; LLOQ, lower limit of quantification; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from Ebola virus, Sudan virus, Marburg virus, and Tai Forest virus nucleoprotein; SFU, spot-forming unit.

[a] Responders for ELISA, ELISpot, or intracellular cytokine staining 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.

[b] ELISpot on frozen peripheral blood mononuclear cells shown as number of SFUs per million cells.

Figure. Ebola Glycoprotein–Specific Antibody Responses for Vaccine Recipients in Randomized Groups at 1 Year (Day 360)



Day 1 is baseline, the day of first vaccination. For numbers of participants included at each time point, see Table. Error bars indicate 95% CIs; Ad26.ZEBOV, adenovirus-type 26 vector vaccine encoding Ebola glycoprotein; MVA-BN-Filo, modified vaccinia Ankara vector vaccine, encoding glycoproteins from the Ebola, Sudan, Marburg, and Tai Forest virus nucleoprotein.

informed consent. The trial was performed in Oxford, United Kingdom, enrolling 87 healthy participants aged 18 to 50 years from December 2014. Twelve-month follow-up was completed March 2016. Seventy-two participants were randomized to 4 groups, each with 18 participants (3 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 days 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 events. 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** for definitions). The number of CD4+ and CD8+ T cells and their cytokine expression patterns were assessed by intracellular cytokine staining, as exploratory outcomes. Data analysis was descriptive (SAS [SAS Institute], 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 the Supplement of the original publication).[1]

Results | Of 75 active vaccine recipients, 64 attended follow-up at day 360 (median age, 39 years; women, 66%). Eleven participants withdrew (1-3 per group) and missing data were not imputed. No serious adverse events were recorded from day 240 through day 360.

All of the active vaccine recipients maintained Ebola virus-specific immunoglobulin G responses at day 360 (**Figure**; Table). Vaccine-induced T-cell responses persisted in 60% to 83% of participants receiving Ad26.ZEBOV first followed by MVA-BN-Filo as a booster compared with 69% to 100% of those receiving the reverse regimen (Table). Persistence of the CD8+ and CD4+ responses is shown in the Table.

Discussion | Immunity after heterologous primary and booster vaccination with Ad26.ZEBOV and MVA-BN-Filo persisted at 1 year. Although no correlate of protection has yet been established, Ebola virus glycoprotein-specific antibodies appear to play an important role in immunity.[5] A strategy of preemptive use of an AD26.ZEBOV followed by MVA-BN-Filo immunization schedule in at-risk populations (where durability of immune response is likely to be of primary importance) may offer advantages over reactive use of

single-dose vaccine regimens.[2,6] A limitation is that this study was conducted in a European population. Immune responses may differ in a sub-Saharan African population; these vaccine candidates are being assessed in this region. Additional research is also warranted to explore the persistence of immunity beyond 1 year following immunization and response to booster doses of vaccine.

Rebecca L. Winslow, MRCGP
Iain D. Milligan, MRCP
Merryn Voysey, MBiostat
Kerstin Luhn, PhD
Georgi Shukarev, MD
Macaya Douoguih, MD
Matthew D. Snape, MD

Author Affiliations: Department of Paediatrics, University of Oxford, Oxford, United Kingdom (Winslow, Milligan, Snape); Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom (Voysey); Janssen, Leiden, the Netherlands (Luhn, Shukarev, Douoguih).

Corresponding Author: Matthew D. Snape, MD, Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Headington, OX3 7LE, United Kingdom (matthew.snape@paediatrics.ox.ac.uk).

Author Contributions: Dr Snape and Ms Voysey had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Milligan, Luhn, Shukarev, Douoguih, Snape.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Winslow, Voysey, Snape.
Critical revision of the manuscript for important intellectual content: Winslow, Milligan, Luhn, Shukarev, Douoguih, Snape.
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Supervision: Milligan, Douoguih, Snape.

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COMMENT & RESPONSE

Cranberry Capsules for Bacteriuria Plus Pyuria in Nursing Home Residents

To the Editor The randomized clinical trial[1] of cranberry capsules to prevent bacteriuria plus pyuria among older women in nursing homes found no significant differences between groups. However, 2 features of the study make the results less than definitive.

First, to assess how much an agent reduces recurrent urinary tract infections (UTIs), a population in which most of the participants have had recurrent infections should be targeted. However, about two-thirds (126 of 185) of the women participating in the study had not had a UTI in the year preceding the trial. Moreover, in the absence of the agent, less than one-third had a UTI in the course of the trial.

Second, the sample size was small, and the rate of withdrawals and missing urine tests was high. As a consequence, the confidence interval accompanying the estimated reduction was wide, ranging from a 39% reduction to a 66% increase.

Therefore, the weakness of the evidence should not be misinterpreted as evidence of a lack of benefit. As the study demonstrated, there are formidable logistical obstacles in studying the extent to which cranberry consumption reduces the incidence or prevalence of UTIs in older women in nursing homes. These age- and setting-specific obstacles would be less of an issue in studying the same question in younger women with recurrent UTIs because studies would not have to rely on urine specimens and could achieve greater sample sizes with less effort.

Chloé Anderson, BSc
Kevin McGregor, MSc
James A. Hanley, PhD

Author Affiliations: Department of Food Science and Agricultural Chemistry, McGill University, Ste Anne de Bellevue, Quebec, Canada (Anderson); Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, Quebec, Canada (McGregor, Hanley).

Corresponding Author: James A. Hanley, PhD, Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, 1020 Av des Pins Ouest, Montréal, QC, Canada H3A 1A2 (james.hanley@mcgill.ca).

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