Vaccines against Ebola virus

Navin Venkatraman[a*], Daniel Silman[a], Pedro M. Folegatti[a], Adrian V.S. Hill[a].

Affiliations

[a]*Jenner Institute, University of Oxford, Roosevelt Drive, Oxford, OX3 7DQ, UK.*

Introduction

On 26 December 2013, when an 18-month-old boy in Meliandou, Guinea developed a fatal illness characterized by fever, black stools, and vomiting, the global scientific community were unaware of the significance of what this heralded. The responsible pathogen was later identified as the Zaire species of the Ebola virus and the World Health Organisation (WHO) issued a public announcement on the 23 March 2014, where 49 cases and 29 deaths were officially reported (1). Subsequently, we have witnessed the largest and most devastating outbreak of Ebola virus disease (EVD) resulting in more cases and deaths than all previous outbreaks combined. Ebola first appeared in 1976 in simultaneous outbreaks in Sudan and Democratic Republic of Congo (DRC) and takes its name from the Ebola River in DRC (2, 3). It is a large, negative-strand RNA virus composed of 7 non-segmented genes encoding viral proteins, including a single glycoprotein (GP) (4, 5). The GP comprises two subunits, which appear as trimeric spikes on the virus surface (6). It plays a pivotal role in cell attachment, fusion and cell entry and its broad cellular tropism results in multisystem involvement and associated high mortality (6). Therefore, the GP has become the key antigenic target for the development of vaccines against EVD.

Setting the stage – Ebola vaccine development prior to the 2014 West African outbreak

Commencing soon after the initial identification of the virus, the first attempts at vaccine development used an inactivated whole virus. This approach never progressed to clinical trials due to potential safety concerns, and failure to demonstrate efficacy in the more predictive non-human

primate (NHP) model (7) despite some earlier efficacy in guinea pigs (8). Recognition of the potential of DNA and viral-vectored vaccines during the 1990s resulted in the first pre-clinical studies expressing the envelope GP or nucleocapsid protein (NP) genes of Ebola virus (9, 10). Efficacy against lethal challenge was demonstrated in the 'gold standard' model of cynomolgus macaques when administered singly or in combination prime-boost regimes (10-12).

The first ever human clinical trial to administer an Ebola vaccine in 2003 used a three-plasmid DNA vaccine encoding the transmembrane-deleted (ΔTM) GP from the Zaire and Sudan species as well as NP, which showed that a 3 dose-schedule was safe and immunogenic (13). Second was a replication-defective, recombinant human adenovirus serotype 5 vaccine (rAd5), which encoded GP genes with a point-mutation (PM). A single vaccination successfully induced T cell and humoral responses against the insert though the latter was partially blunted by pre-existing immunity to Ad5 (14). Non-human primate (NHP) studies, ongoing in a similar timeframe to these human clinical trials, showed that ΔTM GP and PM GP antigens conferred inferior protection to wild-type (WT) GP, which subsequently became the focus of vaccine development (11).

The second clinical trial of a DNA Ebola vaccine assessed safety and immunogenicity of constructs encoding WT GP from Ebola virus Zaire (EBOV) and Sudan (SUDV) species and the Marburgvirus Angola strain (15). Though these trials demonstrated acceptable safety profiles, multiple doses were required and immune responses waned without the administration of a homologous booster dose at 32 weeks (13, 15), and similar findings were shown in a Phase Ib study in Uganda (16).

Strategies to circumvent pre-existing immunity to Ad5 resulted in exploration of the use of serotypes that seldom circulate in humans (e.g. Ad26 and Ad35) and chimpanzee adenoviruses, which have a low human seroprevalence. Promising efficacy data in NHP models showed that rAd26-GP at a dose of 10^{12} viral particles (vp) when given as a single-shot, resulted in 75% efficacy against EBOV challenge. In the same study, a 4 week heterologous prime-boost regime with rAd26-GP/rAd35-GP resulted in 100% efficacy when macaques were challenged 4 weeks post-boost (17). Both

recombinant chimpanzee adenovirus serotype 3 (ChAd3), a subgroup C adenovirus with properties similar to those of Ad5, and serotype 63 (ChAd63) vectors were also considered for development of a new Ebola virus vaccine. Both vectors were shown to be safe in human studies evaluating candidate vaccines for other infectious diseases (18, 19). An efficacy study in NHP of the ChAd3 in both monovalent and bivalent preparations expressing EBOV and SUDV GPs demonstrated 100% efficacy against EBOV challenge with no detectable viraemia (20). Furthermore, durable protection to EBOV challenge 10 months after vaccination was observed when a heterologous boosting vaccination of replication deficient modified vaccinia Ankara (MVA) expressing both GPs was administered 8 weeks post ChAd3 prime. This effect was not seen when boosted with either the same vector or ChAd63, which itself had shown limited immunogenicity and protective efficacy. Hence, the ChAd3 vector was favoured for development into an investigational vaccine for human clinical trials. The well recognised ability of MVA vaccines to provide excellent boosting effect in a number of infectious diseases (21, 22) and the durable efficacy observed in the NHP studies (20) provided evidence for the inclusion of a MVA boost vaccine in human clinical trials.

In addition to the above replication-deficient viral vectors, a recombinant, replication-competent vesicular stomatitis virus (rVSV)-based vaccine encoding EBOV GP had also progressed through preclinical development with encouraging efficacy data in NHP primates (23, 24). It had also been successfully administered to one patient on a compassionate basis for post-exposure prophylaxis following a needle stick injury (25).

Ebola vaccine development since the outbreak

Prior to the 2014 West African outbreak there had only been four completed Phase I vaccine clinical trials in the 38 years following the discovery of the virus (13-16). As the outbreak spread rapidly from Guinea to neighbouring countries, Sierra Leone and Liberia with unprecedented number of cases and deaths, the WHO declared this a Public Health Emergency of International Concern (PHEIC) on 8th August 2014, by which point over 900 people had succumbed to the disease. In addition to other

control measures orchestrated by the WHO and local stakeholders, this announcement heralded extraordinary efforts from the global scientific community to accelerate the development of an Ebola vaccine, ideally one for use in an outbreak setting. National and international efforts to provide funding, coordination, regulatory and ethical review support, expert advice and industrial and manufacturing support were initiated at remarkable speed. Funders included the Wellcome Trust, the European Commission, the US National Institutes of Health (NIH), the UK Medical Research Council, Departments for International Development and Health, and the Bill and Melinda Gates Foundation, many of whom introduced accelerated review mechanisms. Regulatory and ethical reviews of clinical trial protocols were accelerated in Europe, north America and Africa. New vaccines were rapidly designed, developed and manufactured in the US, Europe and Asia with trials rapidly initiated in these continents, Africa and Australia. Coordination activities were led by the WHO with strong input from several major vaccine manufacturers, regulators, public health experts and authorities from the affected countries and regions, academics, funders and relevant non-governmental organisations.

The two leading vaccine candidates that entered Phase I clinical studies in centres in three continents were the monovalent and bivalent ChAd3-vectored vaccine and the rVSV-vectored vaccine, both encoding the GP from the Ebola virus. These immediately accessible vaccines, in addition to a multi-valent MVA-vectored vaccine and an Ad26-vectored vaccine had been manufactured to Good Manufacturing Practice standards earlier, at times some years earlier, supported largely by biodefense funding allocated to develop vaccines that would protect better-off populations from a potential bioterrorist attack.

The numerous Ebola vaccines in clinical development have been reviewed extensively previously (6, 26), so we will subsequently focus on lessons learned from this outbreak for vaccine development, its impact on the future of this field and outbreak management. However, a brief summary of all the vaccines in clinical development precedes this perspective.

Chimpanzee adenovirus 3 vectored vaccine

ChAd3-vectored vaccines expressing the Ebola glycoprotein, both in monovalent and bivalent forms, were the first vaccines to be administered to humans as part of this new wave of clinical trials, in the UK, Europe and US (27-29), and subsequently in Mali (30). These trials were based on early positive pre-clinical studies in non-human primates by the Sullivan group at NIH (20) partnered with the Okairos biotechnology company in Italy, subsequently acquired by GSK. Encouraging clinical safety and immunogenicity provided the basis to commence a large Phase III trial in Liberia, eventually amended to a phase II design due to the decline in new cases of EVD (ClinicalTrials.gov NCT02344407). Clinical trials assessing this vaccine in children aged 1-17 years in Nigeria, Mali and Senegal are ongoing (ClinicalTrials.gov NCT02548078). In addition to single shot vaccine assessment, it has been trialed with prime-boost regimes using MVA (30, 31) and Ad26 vectors (ClinicalTrials.gov NCT02495246).

Vesicular stomatitis virus vectored vaccine

Phase I clinical trials with this replicating vectored vaccine commenced across Europe and Africa shortly following initiation of the ChAd3 trials (32-34). Initial safety concerns were raised due to 10-20% of healthy volunteers reporting severe adverse events. Specifically, arthralgia and arthritides of concern, sometimes associated with VSV in synovial fluid were reported in a trial in Geneva. Objective fever was also reported in 25-30% of volunteers (32). However, this vaccine candidate was chosen along with the ChAd3 vector by the WHO to be used in two sequential Phase III randomised trials in Guinea employing an innovative ring vaccination strategy. Both the ChAd3 and rVSV vaccines could not be tested in parallel and the rVSV was chosen to be assessed first, and by the time this assessment was complete there were too few incident cases to evaluate the ChAd3 vaccine. This landmark trial provided the first evidence of an Ebola vaccine which is highly efficacious and could be stockpiled to curtail future outbreaks (35, 36). Detailed analysis of the safety profile in this population is not described; however, the majority of adverse events were mild. The two serious

adverse events deemed to be related to vaccination resolved without sequelae (35). A further study in Switzerland showed dose reduction, despite improving early reactogenicity, resulted in similar levels of arthritis and reduced immunogenicity (33), consistent with a real difference in the incidence of vaccine-induced arthritis between Europe and West Africa.

Modified vaccinia Ankara vectored vaccine

MVA-BN-Filo, a quadrivalent vaccine encoding the GPs from both EBOV and SUDV, and also the GP from Marburg virus and the NP from the Tai forest strain of Ebolavirus, was the first poxvirus vaccine to be tested in clinical trials. It showed the ability to boost cellular and humoral immune responses many-fold, including neutralising antibodies (31). Testing of very short prime-boost regimes, preferable in outbreak settings, encouragingly suggested immune responses observed using schedules with intervals of 1-2 weeks were comparable to the traditional 8 week interval (31).

The first study to assess the monovalent formulation, MVA EBO Z manufactured in a novel immortalised duck retinal cell line, also tested a very short prime-boost interval of one week and again comparable responses to a 4 week interval were observed in UK adults (37).

Human adenovirus vectored vaccines

A human adenovirus vector (Ad26) was first trialed in regimes with MVA-BN-Filo as both a prime and boost vaccination with intervals of 2, 4 and 8 weeks. Safety profiles observed in this study were acceptable and durable cellular and humoral immune responses were observed at 8 months. Though the MVA prime did not induce an initial immune response in contrast to the Ad26 prime, IgG responses observed at 21 days post boost were slightly higher in the MVA-Ad26 group compared to the Ad26-MVA group (38). Ad26 has also been tested in a heterologous prime-boost regime with the ChAd3-vectored vaccine (ClinicalTrials.gov NCT02495246). Further assessment of safety and immunogenicity including durability of Ad26-MVA regimes are ongoing (ClinicalTrials.gov NCT02661464) and a large phase 3 trial of Ad26-MVA vaccination is in progress in Sierra Leone (ClinicalTrials.gov NCT02509494). There has been very little attention to clinical vaccine

development for the Sudan strain of Ebolavirus during the West African outbreak but just recently a phase I trial of a multi-valent Ad26 vector (Ad26.Filo) in a heterologous prime-boost regime with MVA-BN-Filo has commenced (ClinicalTrials.gov NCT02860650).

Recently, a rAd5-vectored vaccine encoding the GP of the 2014 outbreak strain was shown to be safe and highly immunogenic in healthy adults in China and Sierra Leone (39, 40), though higher doses of 1.6×10^{11} vp were required to overcome pre-existing immunity to rAd5 (39). In addition, responses waned after 4 weeks, though homologous boost at 6 months resulted in several-fold higher antibody responses (41).

Other vaccines in the pipeline

The impetus harnessed from the outbreak for Ebola vaccine development still persists and there are a number of other candidates in either pre-clinical or clinical studies. Viral-vectored and virus-like particle-based vaccines are the predominant type of vaccine being assessed and include a Venezuelan equine encephalitis virus (VEEV)-like replicon particle vaccine, a human parainfluenza type 3 (HPIV3) based vaccine, a recombinant cytomegalovirus based vaccine and a recombinant rabies virus based vaccine. Of these, only the HPIV3-based vaccine has entered human clinical trials in the US and is administered intranasally (ClinicalTrials.gov NCT02564575). Virus like particle (VLP) protein-based vaccines have also been in development and one of these candidate vaccines EBOV GP VLP has been tested in a Phase I clinical trial (ClinicalTrials.gov NCT02370589) in which initial results suggest encouraging safety and immunogenicity with a two dose regimen (42).

Ring vaccination strategy

The concept of ring vaccination was developed in the late 1960s and was part of a new strategy from the smallpox eradication campaign. A focus on surveillance and containment was paramount in the achievement of the eradication target as mass vaccination strategies had proven to be insufficient in developing countries (43). Since then, vaccination of contacts and people at risk has been used as a

control strategy during small and contained Varicella, Measles, Mumps and Rubella outbreaks, preventing second and third waves of infection (44). Until the *Ebola ça Suffit!* trial, the evidence behind the strategy in humans was limited solely to reports from the smallpox eradication campaign in the 1970s, a household contact trial of a meningitis vaccine in Nigeria (45) and mathematical modelling approaches.

The results from *Ebola ça Suffit!* demonstrated that conducting efficacy trials under challenging circumstances of epidemics is feasible and similar methods could potentially be applied in other infectious disease epidemics, bearing in mind the epidemiological characteristics of the disease and the surveillance infra-structure in place (36, 46-48). Effectiveness of ring vaccination strategies rely on a vaccine being efficacious soon after a single dose, transmission patterns (e.g. close human contact) a relatively low R_0 (the basic reproduction number, or the average number of cases generated by a single case), duration of the incubation period and most importantly, a well-structured surveillance system where infectious cases are rapidly diagnosed, isolated and contacts vaccinated promptly. Mathematical modelling studies suggest that ring vaccination strategies could effectively contain an outbreak caused by deliberate release of smallpox as a consequence of bioterror, provided contact tracing is feasible and R_0 is low (49-51).

Vaccine mediated immunity and correlates of protection

An immune correlate for a potential vaccine's efficacy can be regarded as a marker of vaccine-induced immunity that would be predictive of protection. This has been highly sought by EVD vaccine developers since the outbreak as such a marker could theoretically support expedited licensing under the FDA's animal rule (52). Despite the acceleration of clinical trials following the PHEIC and exploration of the immune mechanisms (53) that may underpin protection, such a correlate has remained difficult to firmly define (54, 55) with GP specific antibody titres being the most thoroughly examined (52, 56).

Multiple phase 1 trials with humoral immunogenicity outcomes have shown that the rVSV-vectored vaccine effectively induced GP specific antibodies (32-34). The findings of Regules et al amongst healthy volunteers in the US demonstrated the vaccine induced titres against both Zaire-Mayinga and Zaire-Kikwit strain GP that was dose dependent. Similarly, clinical trials administering single dose ChAd3-vectored vaccines demonstrated comparable anti-GP IgG (28), including neutralising activity and this can be significantly increased with a boosting dose of MVA, even at a short interval (31).

Evidence for vaccine mediated humoral protection against EVD has been suggested by NHP efficacy studies (20, 23, 24) and includes analysis by Sullivan et al of antibody levels in sera from vaccinated macaques, which could theoretically define a threshold titre, above which there is 100% survival on challenge (52). The modest level of antibody immunogenicity induced by the rVSV vaccine after 14 days, a time when where was clear evidence of high vaccine efficacy and negligible T cell immunogenicity, suggest that a quite low level of vaccine-induced antibody may suffice for protection. This appears to conflict with the much higher level (perhaps 10-fold) of antibody required for efficacy in NHP studies (52). A possible resolution is that the level of antibody required to protect against the very large challenge inoculum used in NHP trials is much greater than that required for protection against natural infection in West Africa.

One study with the rVSV-vectored vaccine has also shown depletion of CD4+ T cells during vaccination is associated with blunted antibody response and subsequent loss of protective efficacy (56). The emerging potential of antibody therapeutics in EVD also suggests that strong humoral immunogenicity could contribute to vaccine efficacy as demonstrated by the reversal in viral load seen following the use of monoclonal ZMapp therapy in the patient treated at the Royal Free Hospital, London (57). Assessment of infected individuals in a 1996 outbreak revealed early and increasing levels of IgG amongst survivors compared to those that succumbed (58) and robust early phase antibody responses have also been demonstrated in survivors of the recent outbreak (54). Assays to qualitatively assess antibodies such as neutralization capacity (PsVNA) and antibody

dependent cellular cytotoxicity (ADCC) are frequently included in clinical trials but there is yet to be a clear link with protection from NHP studies (52).

Doubts about the association of anti-glycoprotein titres with protection had lingered from the early negative experimental evidence of passive antibody therapy in animals and humans (57), particularly polyclonal and convalescent plasma preparations. It has also been suggested that until specific antibodies can be clearly mechanistically linked to viral clearance, observed humoral responses may reflect the influence of other aspects of the immune system including HLA type and T cell activation (52) – i.e. falling short of accurately predicting protective immunity themselves, although these arguments appear unpersuasive to many given the evidence of strong virus neutralisation *in vitro*.

The demonstration of strong cellular immunogenicity in human clinical trials of ChAd3 EBOV, particularly when boosted with MVA is interesting (31) as this combination showed durable protection in a 10 month NHP challenge study, associated high absolute frequency (but lower proportion) of TNF- and IFN-γ–coproducing effector cells within the total CD8+ T cell memory pool compared to less protective prime only and ChAd/ChAd schedules (20). Furthermore, CD8+ T cell depletion data in NHP studies after vaccination with the rAd5 vaccine indicated that CD8+ T cells were clearly contributing to protection (59). Transcriptomic analysis of peripheral blood taken from patients in the recent outbreak demonstrated a significant increase in the CD8+ memory T cell signature in survivors in comparison to those with fatal outcome (60).

The challenges for the next period of EVD vaccine development include quantifying the strength of association between specific immune responses and survival, with large efficacy trials such as the outbreak ring vaccination study offering a logical setting to explore this. Of particular interest for the control of future outbreaks, consideration should be given to the identification of markers that correlate with protection that is durable and cross reactive against different Ebolavirus species and strains.

Lessons learned and future perspectives

The PHEIC related to Ebola in West Africa was lifted on 29 March 2016. A total of 28,616 confirmed, probable and suspected cases have been reported in Guinea, Liberia and Sierra Leone, with 11,310 deaths. Amongst the survivors, the prolonged persistence of virus in some bodily fluids like semen (61) and breast milk (62) and the chance of recurrence due to persistence in sites of 'immune privilege' is well documented (63). This in itself poses an ongoing public health concern as the risk of sexual transmission remains a possibility and active measures such as encouraging safe sexual practices with regular semen testing still remains important in order to prevent a resurgence of the outbreak (64). Even in the absence of further human-to-human transmission from this outbreak, it is likely that we will see another outbreak of EVD. We do not know where in Africa this will occur and which species will be responsible. There is an imperative that the lessons learnt from an outbreak of the magnitude that we have witnessed recently, are harnessed to ensure future outbreaks do not reach such a scale.

The accelerated development of a number of vaccine candidates that has just occurred due to a coordinated, collaborative global effort involving a number of stakeholders and agencies is remarkable. We have significant amount of Phase I and Phase II data on a number of vaccines in both Caucasian and African populations. Real-time monitoring and sharing of safety, and to a lesser extent, immunogenicity data led to rapid progress through the pipeline, resulting in the initiation of Phase III trials in West Africa within months after they were first administered to humans. The use of a ring vaccination strategy and the innovative trial design used in Guinea provided us with the first vaccine to be highly efficacious in an outbreak setting. However, there are a number of safety concerns with this replication-competent rVSV-vectored vaccine and whether this vaccine will be licensed in the near future is unclear.

Licensing a vaccine that can be manufactured, stockpiled and rapidly deployed in an outbreak setting remains an unmet goal. The necessity for rapid induction of protective immune responses suggests a

single shot vaccine would be preferable though the demonstrated immunogenicity of prime-boost intervals as short as one week in heterologous regimes offers great encouragement for their deployability, particularly as vaccines providing durable immunity. Of further interest is the first use of an immortalised duck retinal cell line for the production of an MVA-vectored vaccine which gives capacity for greater process control, very large scale manufacturing, high production yields and lower cost of goods compared to other MVA production technology (65).

Closing remarks

The devastating recent outbreak of Ebola led to a clear demonstration of the potential of accelerated vaccine development, capable of protecting high-risk populations much more rapidly than is generally possible. The outbreak has also reconfirmed that it is of particular interest to the scientific community to define immunological correlates of vaccine protection. This can help assess durability of vaccine efficacy against potential future outbreaks of genetically diverse strains and also assists progression of new vaccine candidates in development, possibly facilitating licensing under the FDA animal rule. During this outbreak, disproportionately high numbers of healthcare workers lost their lives in the line of duty (66), emphasizing the need for rapidly deployable protective vaccines to high risk groups and this has been reflected in the strong focus on single dose and short interval prime-boost regimens. Most of the clinical trials to date have been done in adults and we are now seeing data generated in children and HIV-positive subjects (ClinicalTrials.gov NCT02564523). The work of vaccine developers during this public health emergency has also taken place concurrently with valuable research relating to a range of disease control measures including therapeutic interventions (67, 68). These studies offered great insight to vaccine scientists regarding conducting clinical trials in resource-poor countries with limited infrastructure, emphasising the benefits of research collaboration and open communication that should be and were evident during a time of such urgent need.

Conflict of Interest Statement

All authors declare no conflicts of interest.

Contributors

NV, DS and PMF wrote the first draft of the manuscript with subsequent revisions made by all co-authors. All co-authors reviewed the submitted paper.

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References

1. WHO. Origins of the 2014 Ebola epidemic 2015 [Available from: http://who.int/csr/disease/ebola/one-year-report/virus-origin/en/.
2. Johnson KM, Lange JV, Webb PA, Murphy FA. Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. Lancet (London, England). 1977;1(8011):569-71.
3. Pattyn S, van der Groen G, Jacob W, Piot P, Courteille G. Isolation of Marburg-like virus from a case of haemorrhagic fever in Zaire. Lancet (London, England). 1977;1(8011):573-4.
4. Regnery RL, Johnson KM, Kiley MP. Virion nucleic acid of Ebola virus. Journal of virology. 1980;36(2):465-9.
5. Sanchez A, Kiley MP, Holloway BP, Auperin DD. Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. Virus research. 1993;29(3):215-40.
6. Wang Y, Li J, Hu Y, Liang Q, Wei M, Zhu F. Ebola vaccines in clinical trial: The promising candidates. Human vaccines & immunotherapeutics. 2016:1-16.
7. Geisbert TW, Pushko P, Anderson K, Smith J, Davis KJ, Jahrling PB. Evaluation in nonhuman primates of vaccines against Ebola virus. Emerging infectious diseases. 2002;8(5):503-7.
8. Lupton HW, Lambert RD, Bumgardner DL, Moe JB, Eddy GA. Inactivated vaccine for Ebola virus efficacious in guineapig model. Lancet (London, England). 1980;2(8207):1294-5.
9. Vanderzanden L, Bray M, Fuller D, Roberts T, Custer D, Spik K, et al. DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. Virology. 1998;246(1):134-44.
10. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. Nature. 2000;408(6812):605-9.
11. Sullivan NJ, Geisbert TW, Geisbert JB, Shedlock DJ, Xu L, Lamoreaux L, et al. Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. PLoS medicine. 2006;3(6):e177.
12. Sullivan NJ, Geisbert TW, Geisbert JB, Xu L, Yang ZY, Roederer M, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. Nature. 2003;424(6949):681-4.
13. Martin JE, Sullivan NJ, Enama ME, Gordon IJ, Roederer M, Koup RA, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clinical and vaccine immunology : CVI. 2006;13(11):1267-77.
14. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine. 2010;29(2):304-13.
15. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. The Journal of infectious diseases. 2015;211(4):549-57.
16. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, et al. Safety and immunogenicity of Ebola virus and Marburg virus glycoprotein DNA vaccines assessed separately and concomitantly in healthy Ugandan adults: a phase 1b, randomised, double-blind, placebo-controlled clinical trial. Lancet (London, England). 2015;385(9977):1545-54.
17. Geisbert TW, Bailey M, Hensley L, Asiedu C, Geisbert J, Stanley D, et al. Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge. Journal of virology. 2011;85(9):4222-33.
18. Barnes E, Folgori A, Capone S, Swadling L, Aston S, Kurioka A, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Science translational medicine. 2012;4(115):115ra1.

19. O'Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. The Journal of infectious diseases. 2012;205(5):772-81.
20. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med. 2014;20(10):1126-9.
21. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, et al. Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. Nature communications. 2013;4:2836.
22. Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. Science translational medicine. 2014;6(261):261ra153.
23. Jones SM, Feldmann H, Stroher U, Geisbert JB, Fernando L, Grolla A, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nature medicine. 2005;11(7):786-90.
24. Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, Reed DS, Feldmann F, Grolla A, et al. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. Vaccine. 2008;26(52):6894-900.
25. Gunther S, Feldmann H, Geisbert TW, Hensley LE, Rollin PE, Nichol ST, et al. Management of accidental exposure to Ebola virus in the biosafety level 4 laboratory, Hamburg, Germany. The Journal of infectious diseases. 2011;204 Suppl 3:S785-90.
26. Sridhar S. Clinical development of Ebola vaccines. Therapeutic advances in vaccines. 2015;3(5-6):125-38.
27. De Santis O, Audran R, Pothin E, Warpelin-Decrausaz L, Vallotton L, Wuerzner G, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectored Ebola vaccine in healthy adults: a randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. The Lancet Infectious diseases. 2016;16(3):311-20.
28. Ledgerwood JE, DeZure AD, Stanley DA, Coates EE, Novik L, Enama ME, et al. Chimpanzee Adenovirus Vector Ebola Vaccine. The New England journal of medicine. 2017;376(10):928-38.
29. Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede EB, Payne R, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine - Preliminary Report. The New England journal of medicine. 2015. Available from: http://www.nejm.org/doi/suppl/10.1056/NEJMoa1411627/suppl_file/nejmoa1411627_prelim.pdf
30. Tapia MD, Sow SO, Lyke KE, Haidara FC, Diallo F, Doumbia M, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: a phase 1, single-blind, randomised trial, a phase 1b, open-label and double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. The Lancet Infectious diseases. 2016;16(1):31-42.
31. Ewer K, Rampling T, Venkatraman N, Bowyer G, Wright D, Lambe T, et al. A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA. The New England journal of medicine. 2016;374(17):1635-46.
32. Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe. The New England journal of medicine. 2016;374(17):1647-60.
33. Huttner A, Dayer JA, Yerly S, Combescure C, Auderset F, Desmeules J, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. The Lancet Infectious diseases. 2015;15(10):1156-66.
34. Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Hu Z, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine. The New England journal of medicine. 2017;376(4):330-41.
35. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results

from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit!). Lancet (London, England). 2017;389(10068):505-18.
36. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet (London, England). 2015;386(9996):857-66.
37. Venkatraman N, Sridhar S, Wright D, Bowyer G, Powlson J, Rampling T et al., Safety and immunogenicity of the heterologous prime-boost Ebola virus vaccine regimen - ChAd3-EBO Z followed by MVA-EBO Z in healthy UK adults. European Congress of Clinical Microbiology and Infectious Diseases; 2016 11 April 2016; Amsterdam. Available from: https://www.escmid.org/escmid_publications/escmid_elibrary
38. Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. Jama. 2016;315(15):1610-23.
39. Zhu FC, Hou LH, Li JX, Wu SP, Liu P, Zhang GR, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet (London, England). 2015;385(9984):2272-9.
40. Zhu FC, Wurie AH, Hou LH, Liang Q, Li YH, Russell JB, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet (London, England). 2017;389(10069):621-8.
41. Li JX, Hou LH, Meng FY, Wu SP, Hu YM, Liang Q, et al. Immunity duration of a recombinant adenovirus type-5 vector-based Ebola vaccine and a homologous prime-boost immunisation in healthy adults in China: final report of a randomised, double-blind, placebo-controlled, phase 1 trial. The Lancet Global health. 2017;5(3):e324-e34.
42. Novavax. Novavax Ebola/Makona Glycoprotein (GP) Nanoparticle Vaccine Candidate Update: NHP and Clinical Data 2015 [Presentation]. Available from: http://novavax.com/download/files/presentations/Novavax_EBOV_GP_Vaccine_2015_07_21_FINAL.pdf.
43. Strassburg MA. The global eradication of smallpox. American Journal of Infection Control. 1982;10(2):53-9.
44. CDC. Manual for the surveillance of vaccine-preventable diseases. Atlanta: Centers for Disease Control and Prevention; 2012.
45. Greenwood BM, Hassan-King M, Whittle HC. Prevention of secondary cases of meningococcal disease in household contacts by vaccination. British medical journal. 1978;1(6123):1317-9.
46. The ring vaccination trial: a novel cluster randomised controlled trial design to evaluate vaccine efficacy and effectiveness during outbreaks, with special reference to Ebola. BMJ (Clinical research ed). 2015;351:h3740.
47. Kucharski AJ, Eggo RM, Watson CH, Camacho A, Funk S, Edmunds WJ. Effectiveness of Ring Vaccination as Control Strategy for Ebola Virus Disease. Emerging infectious diseases. 2016;22(1):105-8.
48. Merler S, Ajelli M, Fumanelli L, Parlamento S, Pastore YPA, Dean NE, et al. Containing Ebola at the Source with Ring Vaccination. PLoS neglected tropical diseases. 2016;10(11):e0005093.
49. House T, Hall I, Danon L, Keeling MJ. Contingency planning for a deliberate release of smallpox in Great Britain--the role of geographical scale and contact structure. BMC infectious diseases. 2010;10:25.
50. Kretzschmar M, van den Hof S, Wallinga J, van Wijngaarden J. Ring vaccination and smallpox control. Emerging infectious diseases. 2004;10(5):832-41.

51. Porco TC, Holbrook KA, Fernyak SE, Portnoy DL, Reiter R, Aragon TJ. Logistics of community smallpox control through contact tracing and ring vaccination: a stochastic network model. BMC public health. 2004;4:34.
52. Sullivan NJ, Martin JE, Graham BS, Nabel GJ. Correlates of protective immunity for Ebola vaccines: implications for regulatory approval by the animal rule. Nat Rev Microbiol. 2009;7(5):393-400.
53. McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, et al. Biomarker correlates of survival in pediatric patients with Ebola virus disease. Emerging infectious diseases. 2014;20(10):1683-90.
54. McElroy AK, Akondy RS, Davis CW, Ellebedy AH, Mehta AK, Kraft CS, et al. Human Ebola virus infection results in substantial immune activation. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(15):4719-24.
55. McElroy AK, Spiropoulou CF. Biomarkers for understanding Ebola virus disease. Biomarkers in medicine. 2014;8(9):1053-6.
56. Marzi A, Engelmann F, Feldmann F, Haberthur K, Shupert WL, Brining D, et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(5):1893-8.
57. Zeitlin L, Whaley KJ, Olinger GG, Jacobs M, Gopal R, Qiu X, et al. Antibody therapeutics for Ebola virus disease. Current opinion in virology. 2016;17:45-9.
58. Baize S, Leroy EM, Georges-Courbot MC, Capron M, Lansoud-Soukate J, Debre P, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nature medicine. 1999;5(4):423-6.
59. Sullivan NJ, Hensley L, Asiedu C, Geisbert TW, Stanley D, Johnson J, et al. CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nature medicine. 2011;17(9):1128-31.
60. Liu X, Speranza E, Munoz-Fontela C, Haldenby S, Rickett NY, Garcia-Dorival I, et al. Transcriptomic signatures differentiate survival from fatal outcomes in humans infected with Ebola virus. Genome biology. 2017;18(1):4.
61. Sissoko D, Duraffour S, Kerber R, Kolie JS, Beavogui AH, Camara AM, et al. Persistence and clearance of Ebola virus RNA from seminal fluid of Ebola virus disease survivors: a longitudinal analysis and modelling study. The Lancet Global health. 2017;5(1):e80-e8.
62. Sissoko D, Keita M, Diallo B, Aliabadi N, Fitter DL, Dahl BA, et al. Ebola Virus Persistence in Breast Milk After No Reported Illness: A Likely Source of Virus Transmission From Mother to Child. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(4):513-6.
63. Jacobs M, Rodger A, Bell DJ, Bhagani S, Cropley I, Filipe A, et al. Late Ebola virus relapse causing meningoencephalitis: a case report. Lancet (London, England). 2016;388(10043):498-503.
64. Thorson A, Formenty P, Lofthouse C, Broutet N. Systematic review of the literature on viral persistence and sexual transmission from recovered Ebola survivors: evidence and recommendations. BMJ open. 2016;6(1):e008859.
65. Jordan I, Vos A, Beilfuss S, Neubert A, Breul S, Sandig V. An avian cell line designed for production of highly attenuated viruses. Vaccine. 2009;27(5):748-56.
66. Evans DK, Goldstein M, Popova A. Health-care worker mortality and the legacy of the Ebola epidemic. The Lancet Global health. 2015;3(8):e439-40.
67. Sissoko D, Laouenan C, Folkesson E, M'Lebing AB, Beavogui AH, Baize S, et al. Experimental Treatment with Favipiravir for Ebola Virus Disease (the JIKI Trial): A Historically Controlled, Single-Arm Proof-of-Concept Trial in Guinea. PLoS medicine. 2016;13(3):e1001967.
68. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. The New England journal of medicine. 2016;375(15):1448-56.