Viral vectors as vaccine platforms: from immunogenicity to impact

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Abstract

Viral vectors are the vaccine platform of choice for many pathogens that have thwarted efforts towards control using conventional vaccine approaches. Although the STEP trial encumbered development of recombinant human adenovirus vectors only a few years ago, replication-deficient simian adenoviruses have since emerged as a crucial component of clinically effective prime-boost regimens. The vectors discussed here elicit functionally relevant cellular and humoral immune responses, at extremes of age and in diverse populations. The recent Ebola virus outbreak highlighted the utility of viral vectored vaccines in facilitating a rapid response to public health emergencies. Meanwhile, technological advances in manufacturing to support scale-up of viral vectored vaccines have helped to consolidate their position as a leading approach to tackling 'old' and emerging infections.

Introduction

Recombinant viral vectors are a powerful technology for delivering heterologous antigens that combine the best features of other vaccine modalities, with minimal disadvantages. Their capacity to infect cells and express encoded antigens that may be shed into the extracellular milieu or directed to host intracellular processing pathways ensures highly efficient induction of both humoral and cytotoxic (CD8+) T cell responses. This provides a key advantage over subunit vaccines, since CD8+ T cells are essential for the elimination of intracellular pathogens. Viral vectors have intrinsic adjuvant properties, as they express diverse pathogen-associated molecular patterns (PAMPs) which activate innate immunity. Targeted gene deletion is a widely used strategy to reduce or eliminate the replicative capacity of viral vectors, which ensures safety for human use without loss of potency. However, some replication-competent viral vectors can also be given safely and may provide equivalent potency at lower doses. The main drawback of viral vector vaccines is that they may be rendered ineffective by pre-existing or de novo adaptive immune responses to antigenic targets within the vector itself. Strategies to overcome this include the use of higher doses, tolerability permitting, and heterologous prime-boost vaccine regimens.

The~~if~~ development of viral vectors as vaccine platforms has continued unabated, in response to the need for new or improved vaccines against known and emerging pathogens. Two viral vectored vaccines are now licensed for human use and others are likely to follow, as the utility of this technology for a rapid response to global health threats is now clearly recognised. We highlight here the most significant progress in the development of viral vectored vaccines in the past five years and the steps taken to address obstacles to their deployment, together with important insights into mechanisms of protective immunity gained from clinical trials.

Pushing the boundaries: immunogenicity across diverse infectious diseases, populations and age groups

Proof of concept for heterologous prime-boost vaccinations was first demonstrated over a decade ago, with experimental vaccines for malaria using DNA and Modified Vaccinia Virus Ankara (MVA) vectors [1,2]. These early promising results have since been eclipsed by the success of regimens incorporating recombinant adenoviruses as the priming vaccine, thanks to their vastly superior capacity to induce potent cellular and humoral responses. Pre-existing vector-specific immunity, the main drawback of human adenoviruses, has been

effectively circumvented by substitution with replication-deficient (E1-deleted) chimpanzee adenoviruses (ChAds). ChAd63, the first simian adenovirus vector to enter clinical trials, has now been tested in ~1500 individuals [3] (Table 1). A critical result was the finding that prime-boost immunisations with ChAd63- and MVA-vectored P. falciparum malaria vaccines targeting the pre-erythrocytic stage conferred significant protection against both controlled human malaria infection in naïve volunteers and natural infection in malaria-exposed adults. Protection was correlated with high frequencies of antigen-specific $CD8^+$ T cells [4,5]. Following this, spectacular immunogenicity of other ChAd (ChAd63, ChAd3, PanAd3, ChAdOx1) prime / MVA boost regimens in humans has been demonstrated for HIV-1, hepatitis C virus (HCV), influenza and respiratory syncytial virus (RSV) immunogens [6–9] (Table 1).

These studies have been conducted largely in healthy young adults to minimise risk in early stage trials. Encouragingly, a recombinant MVA vaccine was shown to induce comparable cellular immune responses to influenza antigens in adults aged >65 years, a target group in which poor immunogenicity is frequently observed [10]. This is an important finding, as alternative approaches have included higher doses or potent adjuvants, both of which carry the risk of unacceptable reactogenicity [11,12]. Indeed, MVA may provide sufficient intrinsic adjuvant effect, as humoral and cellular responses to licensed (split virion) vaccines were enhanced when co-administered with the MVA-NP+M1 vaccine [13].

Promising results have also emerged from studies in children <5 years, a key population for which malaria and tuberculosis vaccines are urgently needed. In the past decade, MVA has been extensively evaluated in infants, young children and adolescents as a candidate vaccine in prime-boost regimens for *P. falciparum* malaria, tuberculosis and HIV-1, with no associated safety concerns [14–17]. It was recently demonstrated for the first time that ChAd prime-MVA boost regimens can elicit antigen-specific CD8+ T cells in infants as young as 1 week old at similar frequencies to those described in adults (Afolabi M. *et al*., abstract no. 363, 64th Annual Meeting of the American Society of Tropical Medicine and Hygiene, 2015). In addition, IgG antibody titres were 20-fold higher in 10 week-old infants than in adults in the same population. The tuberculosis vaccine candidate, MVA85A, was found to boost antigen-specific CD4+ T cells in BCG-vaccinated infants; unfortunately this did not translate to protective efficacy when assessed in a proof-of-concept trial in South Africa [16]. As the magnitude of 85A-specific T cell responses was lower than in adult vaccinees, these

results highlighted the need to assess more potent viral vectors. Human adenovirus 35, a rare serotype, has since been tested as a boosting vector for delivery of Mycobacterium tuberculosis antigens in BCG-vaccinated infants in the same population. A two-dose regimen of this vaccine not only induced polyfunctional antigen-specific CD4+ T cells but also CD8+ T cells [18].

Viral vectored vaccines move into the fast lane

The 2014 West African Ebola virus outbreak accelerated viral vectored vaccines through safety and efficacy testing in transcontinental consortia to identify best-in-class vaccines with unprecedented speed (Figure 1). Replication-competent vesicular stomatitis virus has taken centre stage as a recombinant Zaire Ebola glycoprotein vaccine (VSV-ZEBOV) following Phase III clinical trial results from endemic areas that demonstrated remarkable efficacy and impressive antibody titres [19]. However, long-term safety post-vaccination is uncertain, particularly given the finding of VSV viraemia in almost all, and secondary arthritis in up to 22% of vaccinees [20]. In contrast, the replication-deficient ChAd3 EBO Z GP vaccine has a less reactogenic adverse event profile and induces durable $CD8^+$ T cell responses as well as neutralising antibodies [21]. Boosting with an MVA Ebola vaccine with different intervals between priming and boosting immunisations affects the kinetics and magnitude of induced immunity, suggesting that different regimens could be tailored for use in outbreak control or preventative mass vaccination campaigns (Figure 2).

If approved, VSV-ZEBOV will be the third viral vectored vaccine to be licensed. The Japanese encephalitis (JE) vaccine ChimeriVax-JE (IMOJEV®) and the tetravalent dengue vaccine, Dengvaxia® comprise a Yellow Fever virus (YFV) encoding two JE viral proteins or four dengue (premembrane and envelope) proteins, respectively. ChimeriVax-JE was first licensed in 2010 in Australia and had an excellent safety and immunogenicity profile in children ≥12 months [22]. Dengvaxia® was recently licensed in Mexico and Brazil in children and adults aged 9-45 years, following demonstration of serotype-specific efficacy of 42-78% (up to 95% against severe disease) in phase III trials [23–25].

Towards identification of functionally relevant immune responses

The systemic T cell responses elicited by viral vectored vaccines are of sufficiently high magnitude to enable a dissection of the qualitative aspects that may be important for

protective immunity. A dominant effector memory phenotype is evident in antigen-specific T cell populations induced by recombinant adenoviruses and poxviruses across vector serotypes, vaccine antigens and animal species [26][7][3]. Phenotypic analyses are now routinely complemented with unbiased systems biology approaches, which were instrumental in identifying predictive gene signatures of efficacy for the yellow fever vaccine [27]. These have revealed an important role of adenovirus-driven innate immune signalling in the induction of potent CD8+ T cell responses which is dominated by type 1 interferons, together with evidence of a highly conserved transcriptional programme of CD8+ T cell differentiation similar to memory responses evoked by persistent viruses [28,29].

Attenuated replicative capacity and lack of persistence in vivo are major determinants of the safety of poxviral vectored vaccines. However, an undesirable consequence of this is the short duration of transgene expression, which may result in ineffective immune responses against persistent pathogens such as human immunodeficiency virus (HIV-1). By contrast, a vaccine deploying a persistent Rhesus CMV vector conferred long-term control and ultimately clearance of simian immunodeficiency virus (SIV) in 60% of vaccinated animals, providing the first evidence that a T cell-based vaccine strategy could abort an AIDS virus infection [30]. Key differences from 'conventional' viral vectored vaccines that induce anamnestic T cell responses and have so far failed to provide long-term protection against HIV-1/SIV were that this CMV-SIV vaccine sustained a long-term effector memory T cell response which was exceptionally broad and comprised non-canonical T cells [31,32]. Whether this can be recapitulated in humans is uncertain but efforts are underway to identify CMV vectors that are sufficiently attenuated for safe human use.

Experience with a ChAd3-vectored Ebola vaccine candidate highlighted the capacity of recombinant adenovirus vaccines to elicit neutralising antibodies to the Mayinga Ebolavirus strain of a similar magnitude to the replication-competent VSV vaccine [21]. Replication-competent adenoviral vectors can improve the immunogenicity of licensed subunit vaccines when used to prime antibody responses against, for example, avian (H5N1) influenza, as was reported for an oral adenovirus type 4-vectored vaccine [51]. Encouraging data are emerging for other viral vectored vaccines, including ChAd63/MVA RH5, which protected *Aotus* monkeys from malaria and elicited parasite growth inhibitory activity in vitro and PanAd3/MVA-RSV, which elicited RSV-specific neutralising activity in adults [33][9][34]. In the macaque model, human adenovirus 26 encoding SIV Gag/Pol/Env followed by Env

protein-in-adjuvant boost protected 50% of vaccinated animals against repeated mucosal heterologous SIV challenge; protection was correlated with multiple Env-specific Fc-mediated antibody functions [35].

Until recently, poxvirus vectors were not considered to be a robust platform for induction of transgene-specific antibodies. However, a post-hoc analysis of the Rv144 trial, which evaluated a recombinant canarypox HIV-1 prime / Env subunit boost regimen, showed that non-neutralising Env V1/V2-specific antibodies may have contributed to protection from HIV-1 acquisition [36].

In a step change from traditional vaccinology, adeno-associated viruses (AAV) are being evaluated as vectors for delivery of genes encoding viral entry inhibitors to prevent HIV-1 infection. AAV-expressed broadly neutralising antibodies or receptor-blocking molecules persisted in vivo over several months and conferred protection against repeated simian/human immunodeficiency virus challenge in non-human primates [37,38]. The isolation of more than 100 novel serotypes and the possibility of cell-free production using directed evolution make AAVs an attractive candidate for antibody-gene delivery [39].

Fulfilling potential: technical advances supporting commercially viable manufacturing and deployment

Limited capacity to deliver vaccines at scale and in resource-limited settings is a perceived disadvantage of viral vectored vaccines but technologies to address this are already in place for replication-deficient adenoviruses [40]. Viral vector production for pre-clinical studies is increased by repression of the transgene during production, typically by use of a cell line that constitutively expresses a tetracyline repressor. However such cell lines were previously not GMP compliant. Procell92® is a Good Manufacturing Practice (GMP)-compliant cell line that incorporates a tet repressor expression cassette to enable efficient virus production [41]. ChAd-vectored Leishmania and hepatitis C vaccine candidates have been produced in this cell line and are entering first-in-human trials (EudraCT 2012-005596-14; EudraCT 2016-000983-41).

Until recently, recombinant MVA production was dependent entirely on propagation in primary chicken embryo fibroblasts derived from pathogen-free eggs, which has limited scalability. A novel duck retinal cell line, AGE1.CR.pIX, is now available and has been adapted

to grow in serum-free medium [42]. Yields are greater than with primary chicken fibroblasts. The first clinical vaccine lot to be produced using this technology was an MVA Ebola Zaire vaccine which entered a phase I clinical trial in 2015 (EudraCT 2015-000593-35).

Deployment of viral vectored vaccines may also be accelerated by improvements in vector immunogenicity to enable reductions in dosing. Fusion of MHC class II invariant chain to HCV or *P. falciparum* immunogens expressed by ChAd and MVA vectors increased the magnitude of the transgene-specific T cell responses by at least two-fold in non-human primates [43,44]. The safety and immunogenicity of this technology will be assessed in a first-in-human trial in 2016 (EudraCT 2016-000983-41). For poxviruses, deletion of viral immunomodulatory genes, use of alternative promoters to optimise transgene expression and combination with adjuvants are being explored (reviewed in [45])[46]. Finally, alternative formulations to eliminate cold-chain requirements, such as nanopatch, which combines needle-free delivery and thermostability, and stabilizing buffers are continually evolving [47][48].

Conclusions and future directions

Given the overlapping epidemiology of many human pathogens, anti-vector immunity remains a potential challenge if the same vectors are to be used for different targets in a given population. However, it is not insurmountable. Typical doses of most adenoviral vectors are capable of readily overcoming naturally acquired anti-vector immunity to the majority of vaccines, with the exception of now little-used common human adenovirus serotypes. Most of the widely used vectors are designed to be replication-incompetent and their immunogenicity is not affected by lack of replication due to anti-vector immunity. Simultaneous administration of multiple vectors encoding different antigens is one possible solution when anti-vector immunity is an issue. Although there is the potential for immune interference, as was observed when ChAd63 and MVA separately encoding the *P. falciparum* blood-stage antigens MSP-1 and AMA-1 were co-administered, it remains to be established whether this phenomenon is specific to particular antigens [49]. Simultaneous contralateral administration of two different vectors, ChAd3 and ChAd63, encoding HCV and HIV-1 immunogens respectively, has recently been assessed in a phase I trial, with excellent safety and no evidence of significant immune interference (Hartnell F. *et al.*, abstract no. LBP507, The International Liver Conference™ 2016, EASL). Furthermore, the pool of viral vectors is expanding; a new ChAd vector from species Human adenovirus E, ChAdOx1 (derived from

Y25) has recently been developed for delivery of Rift Valley Fever (RFV), human influenza and tuberculosis antigens and has proven to be safe and immunogenic in a first-in-human trial [8,50–52] (Table 1). There is also the potential to target several pathogens with a single vaccine, depending on the insertion capacity of the vector (≤6.5 kb in E1-/E3-deleted adenoviruses; >10 kb for orthopoxviruses). However, this requires careful assessment to ensure that vector instability and antigenic competition are minimised. Importantly, the same vector can be reused in the same vaccinee if a suitable dosing interval is allowed: for a malaria antigen in the first clinical trial of a simian adenovirus, reuse of the same vector after 6-12 months allowed effective re-boosting [3]. This likely reflects the poor durability of anti-vector responses that are often outcompeted by responses to the insert in heterologous prime-boost regimens.

In conclusion, in the past five years we have witnessed remarkable progress in viral vectored vaccine development for human infectious diseases. Adenoviral vectors, yellow fever virus vectors and poxvirus vectored vaccines have moved centre-stage and there are many other candidates that may yet surpass them. Given their versatility, potency and safety viral vectors will remain an important tool of modern medicine for the foreseeable future.

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References *(highlighted references shown at end)*

1. McConkey SJ, Reece WH, Moorthy VS, Webster D, Dunachie S, Butcher G, Vuola JM, Blanchard TJ, Gothard P, Watkins K, et al.: **Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans.** *Nat Med.* 2003, **9**:729–735.
2. Moorthy VS, McConkey S, Roberts M, Gothard P, Arulanantham N, Degano P, Schneider J, Hannan C, Roy M, Gilbert SC, et al.: **Safety of DNA and modified vaccinia virus Ankara vaccines against liver-stage P. falciparum malaria in non-immune volunteers**. *Vaccine* 2003, **21**:1995–2002.
3. O'Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, Goodman AL, Edwards NJ, Reyes-Sandoval A, Bird P, et al.: **Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector**. *J. Infect. Dis.* 2012, **205**:772–781.
4. Ewer KJ, O'Hara GA, Duncan CJA, Collins KA, Sheehy SH, Reyes-Sandoval A, Goodman AL, Edwards NJ, Elias SC, Halstead FD, et al.: **Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation**. *Nat Commun* 2013, **4**:2836.
5. Ogwang C, Kimani D, Edwards NJ, Roberts R, Mwacharo J, Bowyer G, Bliss C, Hodgson SH, Njuguna P, Viebig NK, et al.: **Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against Plasmodium falciparum infection in Kenyan adults**. *Sci. Transl. Med.* 2015, **7**:286re5–286re5.
6. Borthwick N, Ahmed T, Ondondo B, Hayes P, Rose A, Ebrahimsa U, Hayton E-J, Black A, Bridgeman A, Rosario M, et al.: **Vaccine-elicited human T cells recognizing conserved protein regions inhibit HIV-1.** *Mol. Ther.* 2014, **22**:464–75.
7. Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, Halliday J, Kelly C, Bowen D, Fergusson J, et al.: **A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory**. *Sci. Transl. Med.* 2014, **6**:261ra153–261ra153.
8. Antrobus RD, Coughlan L, Berthoud TK, Dicks MD, Hill AVS, Lambe T, Gilbert SC: **Clinical Assessment of a Novel Recombinant Simian Adenovirus ChAdOx1 as a Vectored Vaccine Expressing Conserved Influenza A Antigens**. *Mol Ther* 2014, **22**:668–674.
9. Green CA, Scarselli E, Sande CJ, Thompson AJ, de Lara CM, Taylor KS, Haworth K, Del

Sorbo M, Angus B, Siani L, et al.: **Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults**. *Sci. Transl. Med.* 2015, **7**:300ra126–300ra126.

10. Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, Ladell K, Lambe T, Milicic A, Price DA, Hill AVS, et al.: **A T Cell-Inducing Influenza Vaccine for the Elderly: Safety and Immunogenicity of MVA-NP+M1 in Adults Aged over 50 Years**. *PLoS One* 2012, **7**:1–10.
11. A. DC, J. DA, Murray K, Daniel K, John T, Avi C, Richard P, Janet C, John E, Victoria L, et al.: **Efficacy of High-Dose versus Standard-Dose Influenza Vaccine in Older Adults**. *N. Engl. J. Med.* 2014, **371**:635–645.
12. Iob A, Brianti G, Zamparo E, Gallo T: **Evidence of increased clinical protection of an MF59-adjuvant influenza vaccine compared to a non-adjuvant vaccine among elderly residents of long-term care facilities in Italy.** *Epidemiol. Infect.* 2005, **133**:687–693.
13. Antrobus RD, Berthoud TK, Mullarkey CE, Hoschler K, Coughlan L, Zambon M, Hill AVS, Gilbert SC: **Coadministration of Seasonal Influenza Vaccine and MVA-NP+M1 Simultaneously Achieves Potent Humoral and Cell-Mediated Responses**. *Mol Ther* 2014, **22**:233–238.
14. Bejon P, Ogada E, Mwangi T, Milligan P, Lang T, Fegan G, Gilbert SC, Peshu N, Marsh K, Hill AVS: **Extended Follow-Up Following a Phase 2b Randomized Trial of the Candidate Malaria Vaccines FP9 ME-TRAP and MVA ME-TRAP among Children in Kenya**. *PLoS One* 2007, **2**:1–5.
15. Scriba TJ, Tameris M, Mansoor N, Smit E, van der Merwe L, Isaacs F, Keyser A, Moyo S, Brittain N, Lawrie A, et al.: **Modified vaccinia Ankara-expressing Ag85A, a novel tuberculosis vaccine, is safe in adolescents and children, and induces polyfunctional CD4+ T cells**. *Eur. J. Immunol.* 2010, **40**:279–290.
16. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea JE, McClain JB, Hussey GD, Hanekom WA, et al.: **Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial**. *Lancet* 2013, **381**:1021–1028.
17. Greenough TC, Cunningham CK, Muresan P, McManus M, Persaud D, Fenton T, Barker P, Gaur A, Panicali D, Sullivan JL, et al.: **Safety and immunogenicity of recombinant poxvirus HIV-1 vaccines in young adults on highly active antiretroviral therapy.** *Vaccine* 2008, **26**:6883–93.

18. Kagina BMN, Tameris MD, Geldenhuys H, Hatherill M, Abel B, Hussey GD, Scriba TJ, Mahomed H, Sadoff JC, Hanekom WA, et al.: **The novel tuberculosis vaccine, AERAS-402, is safe in healthy infants previously vaccinated with BCG, and induces dose-dependent CD4 and CD8 T cell responses**. *Vaccine* 2014, **32**:5908–5917.
19. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, Carroll MW, Doumbia M, Draguez B, Duraffour S, 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* 2015, **386**:857–866.
20. Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, Yerly S, Dayer J, Kraehling V, Kasonta R, et al.: **Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe — Preliminary Report**. *N. Engl. J. Med.* 2015, doi:10.1056/NEJMoa1502924.
21. Rampling T, Ewer K, Bowyer G, Wright D, Imoukhuede EB, Payne R, Hartnell F, Gibani M, Bliss C, Minhinnick A, et al.: **A Monovalent Chimpanzee Adenovirus Ebola Vaccine — Preliminary Report**. *N. Engl. J. Med.* 2015, doi:10.1056/NEJMoa1411627.
22. Halstead SB, Thomas SJ: **New Japanese encephalitis vaccines: alternatives to production in mouse brain**. *Expert Rev. Vaccines* 2011, **10**:355–364.
23. Capeding MR, Tran NH, Hadinegoro SRS, Ismail HIHJM, Chotpitayasunondh T, Chua MN, Luong CQ, Rusmil K, Wirawan DN, Nallusamy R, et al.: **Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial**. *Lancet* 2014, **384**:1358–1365.
24. Villar L, Dayan GH, Arredondo-García JL, Rivera DM, Cunha R, Deseda C, Reynales H, Costa MS, Morales-Ramírez JO, Carrasquilla G, et al.: **Efficacy of a Tetravalent Dengue Vaccine in Children in Latin America**. *N. Engl. J. Med.* 2014, **372**:113–123.
25. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, Hj Muhammad Ismail HI, Reynales H, Limkittikul K, Rivera-Medina DM, et al.: **Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease**. *N. Engl. J. Med.* 2015, **373**:1195–1206.
26. Quinn KM, Da Costa A, Yamamoto A, Berry D, Lindsay RWB, Darrah PA, Wang L, Cheng C, Kong W-P, Gall JGD, et al.: **Comparative Analysis of the Magnitude, Quality, Phenotype, and Protective Capacity of Simian Immunodeficiency Virus Gag-Specific CD8+ T Cells following Human-, Simian-, and Chimpanzee-Derived Recombinant Adenoviral Vector Immunization**. *J. Immunol.* 2013, **190** :2720–2735.
27. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K,

Deng J, Marzolf B, et al.: **Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans**. *Nat Immunol* 2009, **10**:116–125.

28. Bolinger B, Sims S, Swadling L, O'Hara G, de Lara C, Baban D, Saghal N, Lee LN, Marchi E, Davis M, et al.: **Adenoviral Vector Vaccination Induces a Conserved Program of CD8+ T Cell Memory Differentiation in Mouse and Man**. *Cell Rep.* 2015, **13**:1578–1588.
29. Zak DE, Andersen-Nissen E, Peterson ER, Sato A, Hamilton MK, Borgerding J, Krishnamurty AT, Chang JT, Adams DJ, Hensley TR, et al.: **Merck Ad5/HIV induces broad innate immune activation that predicts CD8+ T-cell responses but is attenuated by preexisting Ad5 immunity**. *Proc. Natl. Acad. Sci.* 2012, **109** :E3503–E3512.
30. Hansen SG, Piatak Jr. M, Ventura AB, Hughes CM, Gilbride RM, Ford JC, Oswald K, Shoemaker R, Li Y, Lewis MS, et al.: **Immune clearance of highly pathogenic SIV infection**. *Nature* 2013, **502**:100–104.
31. Hansen SG, Sacha JB, Hughes CM, Ford JC, Burwitz BJ, Scholz I, Gilbride RM, Lewis MS, Gilliam AN, Ventura AB, et al.: **Cytomegalovirus vectors violate CD8+ T cell epitope recognition paradigms**. *Science (80-.).* 2013, **340**:1237874.
32. Hansen SG, Wu HL, Burwitz BJ, Hughes CM, Hammond KB, Ventura AB, Reed JS, Gilbride RM, Ainslie E, Morrow DW, et al.: **Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E**. *Science (80-.).* 2016, **351**:714–720.
33. Sheehy SH, Duncan CJ, Elias SC, Biswas S, Collins KA, O'Hara GA, Halstead FD, Ewer KJ, Mahungu T, Spencer AJ, et al.: **Phase Ia clinical evaluation of the safety and immunogenicity of the Plasmodium falciparum blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors**. *PLoS One* 2012, **7**:e31208.
34. Douglas AD, Baldeviano GC, Lucas CM, Lugo-Roman LA, Crosnier C, Bartholdson SJ, Diouf A, Miura K, Lambert LE, Ventocilla JA, et al.: **A PfRH5-Based Vaccine Is Efficacious against Heterologous Strain Blood-Stage Plasmodium falciparum Infection in Aotus Monkeys**. *Cell Host Microbe* 2015, **17**:130–139.
35. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, Borducchi EN, Smith KM, Nkolola JP, Liu J, et al.: **Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys**. *Science (80-.).* 2015, **349**:320–324.
36. F. HB, B. GP, Juliana MM, Susan Z-P, D. TG, Munir AS, T. ED, C. MD, Chitraporn K, Ruengpueng S, et al.: **Immune-Correlates Analysis of an HIV-1 Vaccine Efficacy Trial**. *N. Engl. J. Med.* 2012, **366**:1275–1286.

37. Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, An DS, Baltimore D: **Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission**. *Nat Med* 2014, **20**:296–300.

38. Gardner MR, Kattenhorn LM, Kondur HR, von Schaewen M, Dorfman T, Chiang JJ, Haworth KG, Decker JM, Alpert MD, Bailey CC, et al.: **AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges**. *Nature* 2015, **519**:87–91.

39. Muzyczka N, Berns KI: **AAV's Golden Jubilee**. *Mol Ther* 2015, **23**:807–808.

40. Vellinga J, Smith JP, Lipiec A, Majhen D, Lemckert A, van Ooij M, Ives P, Yallop C, Custers J, Havenga M: **Challenges in Manufacturing Adenoviral Vectors for Global Vaccine Product Deployment**. *Hum. Gene Ther.* 2014, **25**:318–327.

41. Vitelli A, Quirion MR, Lo C-Y, Misplon JA, Grabowska AK, Pierantoni A, Ammendola V, Price GE, Soboleski MR, Cortese R, et al.: **Vaccination to Conserved Influenza Antigens in Mice Using a Novel Simian Adenovirus Vector, PanAd3, Derived from the Bonobo <italic>Pan paniscus</italic>**. *PLoS One* 2013, **8**:1–9.

42. Lohr V, Rath A, Genzel Y, Jordan I, Sandig V, Reichl U: **New avian suspension cell lines provide production of influenza virus and MVA in serum-free media: Studies on growth, metabolism and virus propagation**. *Vaccine* 2009, **27**:4975–4982.

43. Capone S, Naddeo M, D'Alise AM, Abbate A, Grazioli F, Del Gaudio A, del Sorbo M, Esposito ML, Ammendola V, Perretta G, et al.: **Fusion of HCV non-structural antigen to MHC Class II associated invariant chain enhances T cell responses induced by vectored vaccines in non-human primates**. *Mol Ther* 2014, **22**:1039–47.

44. Spencer AJ, Cottingham MG, Jenks JA, Longley RJ, Capone S, Colloca S, Folgori A, Cortese R, Nicosia A, Bregu M, et al.: **Enhanced Vaccine-Induced CD8$^+$ T Cell Responses to Malaria Antigen ME-TRAP by Fusion to MHC Class II Invariant Chain**. *PLoS One* 2014, **9**:e100538.

45. García-Arriaza J, Esteban M: **Enhancing poxvirus vectors vaccine immunogenicity**. *Hum. Vaccin. Immunother.* 2014, **10**:2235–2244.

46. Alharbi NK, Spencer AJ, Salman AM, Tully CM, Chinnakannan S k., Lambe T, Yamaguchi Y, Morris SJ, Orubu T, Draper SJ, et al.: **Enhancing cellular immunogenicity of MVA-vectored vaccines by utilizing the F11L endogenous promoter**. *Vaccine* 2016, **34**:49–55.

47. Pearson FE, McNeilly CL, Crichton ML, Primiero CA, Yukiko SR, Fernando GJP, Chen X, Gilbert SC, Hill AVS, Kendall MAF: **Dry-Coated Live Viral Vector Vaccines Delivered by**

Nanopatch Microprojections Retain Long-Term Thermostability and Induce Transgene-Specific T Cell Responses in Mice. *PLoS One* 2013, **8**:e67888.

48. Ghobadloo SM, Balcerzak AK, Gargaun A, Muharemagic D, Mironov GG, Capicciotti CJ, Briard JG, Ben RN, Berezovski M V: **Carbohydrate-Based Ice Recrystallization Inhibitors Increase Infectivity and Thermostability of Viral Vectors**. *Sci. Rep.* 2014, **4**:5903.
49. Sheehy SH, Duncan CJA, Elias SC, Choudhary P, Biswas S, Halstead FD, Collins KA, Edwards NJ, Douglas AD, Anagnostou NA, et al.: **ChAd63-MVA-vectored Blood-stage Malaria Vaccines Targeting MSP1 and AMA1: Assessment of Efficacy Against Mosquito Bite Challenge in Humans**. *Mol Ther* 2012, **20**:2355–2368.
50. Dicks MDJ, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, Hill AVS, Cottingham MG: **A Novel Chimpanzee Adenovirus Vector with Low Human Seroprevalence: Improved Systems for Vector Derivation and Comparative Immunogenicity**. *PLoS One* 2012, **7**:e40385.
51. Warimwe GM, Lorenzo G, Lopez-Gil E, Reyes-Sandoval A, Cottingham MG, Spencer AJ, Collins KA, Dicks MDJ, Milicic A, Lall A, et al.: **Immunogenicity and efficacy of a chimpanzee adenovirus-vectored Rift Valley Fever vaccine in mice**. *Virol. J.* 2013, **10**:1–9.
52. Stylianou E, Griffiths KL, Poyntz HC, Harrington-Kandt R, Dicks MD, Stockdale L, Betts G, McShane H: **Improvement of BCG protective efficacy with a novel chimpanzee adenovirus and a modified vaccinia Ankara virus both expressing Ag85A**. *Vaccine* 2015, **33**:6800–6808.
53. Ewer K et al.: **A Monovalent Chimpanzee Adenovirus Ebola Vaccine boosted with MVA**. *N Engl J Med* 2016, **in press**.
54. Sheehy SH, Duncan CJA, Elias SC, Collins KA, Ewer KJ, Spencer AJ, Williams AR, Halstead FD, Moretz SE, Miura K, et al.: **Phase Ia Clinical Evaluation of the Plasmodium falciparum Blood-stage Antigen MSP1 in ChAd63 and MVA Vaccine Vectors**. *Mol Ther* 2011, doi:http://www.nature.com/mt/journal/vaop/ncurrent/suppinfo/mt2011176s1.html.
55. de Barra E, Hodgson SH, Ewer KJ, Bliss CM, Hennigan K, Collins A, Berrie E, Lawrie AM, Gilbert SC, Nicosia A, et al.: **A Phase Ia Study to Assess the Safety and Immunogenicity of New Malaria Vaccine Candidates ChAd63 CS Administered Alone and with MVA CS**. *PLoS One* 2014, **9**:e115161.
56. Hodgson SH, Ewer KJ, Bliss CM, Edwards NJ, Rampling T, Anagnostou NA, de Barra E,

Havelock T, Bowyer G, Poulton ID, et al.: **Evaluation of the Efficacy of ChAd63-MVA Vectored Vaccines Expressing Circumsporozoite Protein and ME-TRAP Against Controlled Human Malaria Infection in Malaria-Naive Individuals**. *J. Infect. Dis.* 2015, **211** :1076–1086.

57. Hodgson SH, Choudhary P, Elias SC, Milne KH, Rampling TW, Biswas S, Poulton ID, Miura K, Douglas AD, Alanine DGW, et al.: **Combining Viral Vectored and Protein-in-adjuvant Vaccines Against the Blood-stage Malaria Antigen AMA1: Report on a Phase 1a Clinical Trial**. *Mol Ther* 2014, **22**:2142–2154.
58. Hayton E-J, Rose A, Ibrahimsa U, Del Sorbo M, Capone S, Crook A, Black AP, Dorrell L, Hanke T: **Safety and Tolerability of Conserved Region Vaccines Vectored by Plasmid DNA, Simian Adenovirus and Modified Vaccinia Virus Ankara Administered to Human Immunodeficiency Virus Type 1-Uninfected Adults in a Randomized, Single-Blind Phase I Trial**. *PLoS One* 2014, **9**:e101591.
59. Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Muñoz P, Moon JE, Ruck RC, Bennett JW, Twomey PS, et al.: **A Recombinant Vesicular Stomatitis Virus Ebola Vaccine — Preliminary Report**. *N. Engl. J. Med.* 2015, doi:10.1056/NEJMoa1414216.
60. Ledgerwood JE, DeZure AD, Stanley DA, Novik L, Enama ME, Berkowitz NM, Hu Z, Joshi G, Ploquin A, Sitar S, et al.: **Chimpanzee Adenovirus Vector Ebola Vaccine — Preliminary Report**. *N. Engl. J. Med.* 2014, doi:10.1056/NEJMoa1410863.
61. Tapia MD, Sow SO, Lyke KE, Haidara FC, Diallo F, Doumbia M, Traore A, Coulibaly F, Kodio M, Onwuchekwa U, 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**. *Lancet Infect. Dis.* 2016, **16**:31–42.

Highlighted references

*5. In this trial, 67% efficacy against P. falciparum infection was observed in Kenyan adults vaccinated with ChAd63- and MVA-vectored ME-TRAP.

**19. High level of efficacy of a replication-competent viral vectored vaccine against Ebola virus.

**24. Demonstration of efficacy of a chimeric tetravalent dengue vaccine in over 20 thousand children, with a high level of protection against severe disease and against serotypes 3 and 4, in particular.

*28. Demonstrates conserved transcriptional programming induced by adenovirus vectors in mice and humans

*32. Describes unique features of CD8+ T cell responses elicited by Rhesus CMV-vectored SIV vaccine, including those restricted by HLA-E.

*47. Demonstration that the combination of two highly desirable qualities for novel vaccines (thermostability and needle-free) are feasible for viral vectored vaccines, making them highly suitable for cost-effective deployment.

Figure Legends

Figure 1. Time line of MVA EBO Z GP production

MVA EBO Z GP was manufactured in a cell-line not previously used for human vaccine production. Despite being a "first-in-human" study, the vaccine was produced, released and approved for trial use in around six-months. Use of a new cell line for MVA production required additional discussion with the UK regulator (MHRA) regarding suitable release assays (http://www.ox.ac.uk/news/2015-05-13-new-study-ebola-vaccine-candidates).

Figure 2. Effect of prime-boost interval on immune response kinetics and magnitude

Shorter prime-boost intervals induce high frequencies of antigen-specific T cells, similar in magnitude to 3 - 8 week interval regimes. However, peak antibody responses are substantially reduced by shorter intervals, as are functional measures such as neutralising capability [53].

Table 1 Summary of clinical trials of replication-deficient chimpanzee adenovirus-vectored vaccines

Vector	Disease	Insert/Antigen	Trial Phase	Age-group	Country	N*	
ChAd63	Malaria	ME-TRAP	I and II	Adults, children, infants, neonates	UK, Kenya, The Gambia, Burkina Faso, Senegal.	1005	[4][3][54]
		CSP	I and II	Adults	UK, Ireland.	96	[55][56]
		MSP-1	I and II	Adults	UK	65	[49][54]
		AMA-1	I and II	Adults	UK	60	[49][33][57]
		PvDBP	I	Adults	UK	24	NCT01816113
		RH5	I	Adults	UK	24	NCT02181088
		Pfs25	I	Adults	UK	24	NCT02532049
	HIV	HIVconsv (Gag, Pol, Vif, Env)	I	Adults	UK	116	[6][58]NCT02362217 NCT01712425 NCT02425241
ChAd3	HCV	NSmut (NS3, NS4A, NS4B, NS5A, NS5B)	I and II	Adults	UK, USA, Ireland, Switzerland.	446	[7][58]NCT01436357 NCT02362217 NCT02568332
	Ebola	Glycoprotein (Ebolavirus Zaire +/- Sudan)	I, II and III	Adults and children	UK, Switzerland, USA, Senegal, Mali, Uganda, Liberia.	4500**	[19][20][21][59][60][61] NCT02354404 NCT02344407
PanAd3	RSV	F, N, M2-1	I	Adults	UK	70	[9]NCT01805921
ChAOx1	Influenza	NP, M1	I	Adults	UK	87	[8]
	Tuberculosis	85A	I	Adults	UK	42	NCT01829490

For ongoing trials, “N” refers to target enrolment numbers indicated on clinicaltrials.gov, accessed 29th February 2016.

* For Phase II studies, participants receiving placebo are not included in total.

** Excluding the PREVAIL trial in Liberia, which aims to recruit 28170 participants randomised to receive either ChAd3-EBO Z, VSVG-ZEBOV or placebo.