Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Viral vector vaccines
Naina McCann, Daniel O’Connor, Teresa Lambe and Andrew J Pollard

Over the past two years, the SARS-CoV-2 pandemic has highlighted the impact that emerging pathogens can have on global health. The development of new and effective vaccine technologies is vital in the fight against such threats. Viral vectors are a relatively new vaccine platform that relies on recombinant viruses to deliver selected immunogens into the host. In response to the SARS-CoV-2 pandemic, the development and subsequent rollout of adenoviral vector vaccines has shown the utility, impact, scalability and efficacy of this platform. Shown to elicit strong cellular and humoral immune responses in diverse populations, these vaccine vectors will be an important approach against infectious diseases in the future.

Addresses
1 Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Headington, Oxford OX3 7LE, UK
2 NIHR Oxford Biomedical Research Centre, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Corresponding author:
Naina McCann (Naina.mccann@paediatrics.ox.ac.uk)

Immunogenicity

Innate immune response
Using viral vectors as vaccine platforms allows induction of an innate immune response without the need for adjuvant. This response is key for stimulating downstream processing and later adaptive immune responses (Figure 2).

The downstream patterns of signalling from Ad vector recognition involve induction of a proinflammatory response, including cytokine and chemokine production, inducing humoral and cellular responses. Importantly, Ad vectors are able to do this without causing host damage and excess cytokine production. However, the excessive induction of type-I interferons (IFNs) by Ad vectors has been associated with dampened transgene expression and reduced antibody and cellular responses [34,35].

Employing bioinformatic techniques to investigate transcriptional changes induced by viral vectors can give new insight into the activation of innate immune pathways by viral vectors. In a study by Sheerin et al using a mouse immunisation model, Ad vectors induced expression of genes involved in TLR2 stimulation and natural killer (NK) cell activation, whereas modified vaccinia Ankara (MVA) vectors induced expression of type-1 IFN genes [36]. Collingnon et al evaluated cytokine responses and gene expression patterns to characterise innate responses following vaccination with the ChAd155 vector vaccines in preclinical studies. The authors showed that the vaccine induced a bimodal pattern of innate cell-population changes characterised by IFN-associated signatures [37].

Adaptive immune response
The ability of viral vectors to infect host cells, and express heterologous antigen, allows for antigen presentation and
activation of host MHC pathways via direct and cross-presentation, inducing a robust cellular response (Figure 3). The amount and duration of antigen expression correlate with CD8+ T-cell-protective immunity [35]. This potent T-cell activation has led to prior targeting of viral vector vaccines against intracellular pathogens, for example, HIV and malaria where such responses to such vaccines have correlated with protection [38].

Recent work has confirmed the strong and durable CD4+ and CD8+ antigen-specific T-cell responses that are generated following viral vector vaccines against other pathogens such as SARS-CoV-2, Ebola and RSV [4,11,39–41]. The T-cell response following viral vector vaccination appears to be a Th-1-biased response, characterised by IFN-γ and TNF-alpha production [27,41–43]. Strong transgene expression by Ad vector vaccines also allows robust mono- and polyfunctional CD8+ T-cell responses [42,44].

These specific T-cell responses may also contribute to protection and reduction in disease severity. For example, when examining these SARS-CoV-2-specific T-cell responses following acute COVID-19 infection, they appear to inversely correlate with COVID-19 disease severity [45,46]. Additionally, SARS-CoV-2 spike-specific follicular helper T cells correlate with neutralising antibody responses [47]. T-cell epitopes also appear to remain relatively preserved in COVID-19 variants of concern (VoC), leading to limited T-cell escape following infection or vaccination [48–50]. Given that these VoC have significant mutations in spike protein, leading to evasion of the neutralising antibody response [51–53], the ability of Ad vector vaccines to induce a broad cellular response may be important in sustaining protection from SARS-CoV-2.

As described above, although T-cell-mediated immunity plays a role in reducing disease severity, a neutralising antibody response often correlates with protection against infection. High levels of neutralising antibodies are induced by vesicular stomatitis virus (VSV), MVA and Ad viral vector vaccines against Ebola virus [23,27,54,55] and following Ad vector vaccines against SARS-CoV-2 [12,39,42]. When investigating correlates of protection against SARS-CoV-2 following ChAdOx1-nCoV-19 vaccination higher anti-spike IgG, anti-receptor-binding protein IgG and neutralising antibody titres were all associated with lower risk of symptomatic disease [56]. All four Ad viral vector vaccines (Ad26.COV2.S, ChAdOx1-nCoV, Gam-COVID-Vac and Ad5-nCoV) are effective in protecting against symptomatic COVID-19 (66.9%, 66.7%, 91.6% and 57.5%, respectively) [11,12,21,57,58].
Table 1
Currently licensed viral vector vaccines for use in humans.

| Vector class | Vector | Vaccine | Target pathogen | Encoded antigen | Developer | Clinical trials |
|--------------|--------|---------|-----------------|-----------------|-----------|-----------------|
| Adenoviruses | Ad5    | Ad5-nCoV (Convidecia) | SARS-CoV-2 | Spike protein | CanSino Biologics (China) | [3,4] |
|             | Ad5-EBOV | Ebola virus |            | Zaire strain (Makona) of glycoprotein | CanSino Biologics Inc | [5-7] |
|             | Ad26   | Ad26. CoV | SARS-CoV-2 | Pre-fusion- stabilised spike protein | Janssen Pharmaceutical Companies | [8,9] |
|             |        | Sputnik light | SARS-CoV-2 | Spike protein | Gamaleya Research Institute of Epidemiology and Microbiology (Russia) | [10] |
|             | ChAdOx1 | ChAdOx1- nCoV-19 (Covishield, Vaxzevria) | SARS-CoV-2 | Spike protein with tissue plasminogen leader sequence | University of Oxford/AstraZeneca | [11-13] |
|             | VSV    | VSV-EBOV (VSV-ZEBOV, Ervebo) | Ebola virus | Zaire strain (Kikwit 1995) of glycoprotein | Merck | [14,15] |
| Rhabdoviruses | YF 17D | ChimerVax-JE (Imojev) | Japanese encephalitis | Viral envelope (prM and E) of JE strain SA14-14-2 | Sanofi Pasteur | [16-18] |
| Flaviviruses | CYD-TDV (Dengvaxia) | Dengue | prM and E genes of DENV 1-4 | | Sanofi Pasteur | [19,20] |
|             | Gam-COVID-Vac (Sputnik V) | SARS-CoV-2 | Both spike proteins | Sanofi Pasteur | [21,22] |
| Heterologous | Ad5/Ad26 | GamEvac-Combi | Ebola virus | Both glycoproteins | Janssen Pharmaceutical Companies | [23] |
| regimes     | VSV/Ad5 | GamEvac-Combi | Ebola virus | Both glycoproteins | | |
|             | Ad26/MVA | Ad26. ZEBOV (Zabdeno) | Ebola virus | Ad26 — Zaire strain MVA — glycoproteins from the Zaire Ebola virus (Mayinga strain) Sudan virus (Gulu strain) and Marburg virus (Mayinga strain), and the nucleoprotein from the Tai Forest virus | Janssen Pharmaceutical Companies | [24-26] |

Viral vector vaccines utilise the capacity of viruses to infect cells and induce broad immune responses. Heterologous antigens are expressed by the virus, usually from genes engineered into the viral genome, and induce antigen-specific humoral and cellular immune responses. Viral vectors themselves can be replication-deficient, replication-competent or attenuated. Replication of the virus inside cells allows ongoing amplification of the vaccine antigen and improved immunogenicity, but must be balanced against the risk of increased adverse events or even disease in the host, particularly in the immunocompromised, resulting in some preference for use of replication-incompetent vectors.
Induction of innate and adaptive immune responses by adenoviral vector vaccines. Adenoviral binding occurs via the fibre protein of the Ad capsid to infection receptors, such as the coxsackievirus–adenovirus receptor (CAR) and CD46, activating entry to the cell. Secondary attachment is mediated by RGD loops on the penton protein of the Ad capsid to integrins. These binding processes themselves can trigger innate immunity, but it is the pathogen-associated molecular patterns of adenoviruses, which are recognised by cell pattern-recognition receptors (PRRs), for example, toll-like receptors (TLRs). Ad vectors are recognised by TLR2 and TLR4, which are surface receptors, and TLR9, an endosomally located receptor that senses the Ad vector genome in endosomes [29,30]. The binding of lactoferrin, a host defence peptide, to Ad vectors, appears to activate an innate immune response via TLR4-mediated internalisation [31]. Intracellular adaptor proteins, such as MyD88, are vital for TLR signal transduction and induction of antigen-specific T-cell responses via activation of NF-κB transcription factors following Ad vector vaccine [32]. Further, PRRs such as the cytosol DNA sensor cGAS and the receptor RIG-I area are also important for inducing innate immune signals following Ad vector vaccination [33].

Non-neutralising antibodies are also recognised as important mediators of antipathogen immunity and in preclinical studies Fc-mediated functions were shown to contribute to protection against SARS-CoV-2 [59,60] and Ebola [61]. Systems’ serology work has shown that Ad vector vaccines are able to induce antibody-dependent functional activities, including antibody-dependent neutrophil phagocytosis and antibody-dependent monocyte phagocytosis [8]. In a comparison of vaccine responses from phase-I and phase-II studies in humans using different HIV vaccines, Ad viral vectors induced a more potent IgG1 and IgG3 response than pox-virus vectors, leading to higher levels of functional antibody activity, including antibody-dependent cellular phagocytosis [62].

Induction of a mucosal immune response is likely to play an important role in protection against respiratory pathogens. Provine et al showed that in ChAdOx1-nCoV-19-immunised mice, mucosal-associated invariant T cells were induced, which correlated with vaccine-mediated T-cell responses [63]. Mucosal administration of an Ad vaccine may also induce stronger mucosal immune responses. Lapuente et al showed that mice given an intranasal Ad vector vaccine (either Ad19a or Ad5) boost following an intramuscular plasmid DNA or mRNA prime induced high levels of mucosal IgA and lung-resident tissue-resident memory T cells, in addition to systemic responses, leading to enhanced mucosal neutralisation [64]. Human trials of mucosal Ad vector vaccines against SARS-CoV-2 are underway with phase-I
data from an aerosolised Ad5.nCoV vaccine showing two doses elicit a neutralising antibody response similar to one dose of IM injection [65].

**Pre-existing immunity**

Pre-existing immunity against the Ad vector has the potential to reduce immunogenicity and subsequent protective effect of these vaccine vectors [66]. Multiple studies have shown that existing anti-Ad-neutralising antibodies are inversely correlated with immunological response to vaccine vector [3,6,67]. This is particularly relevant with Ad5-based vector vaccines, given their high seroprevalence in some populations [68]. However, repeated doses of Ad26 vector vaccination against HIV are able to boost both cellular and humoral immune responses, despite the presence of high Ad26-neutralising antibodies following prime vaccination [69] and following vaccination with ChAdOx1-nCoV-19-neutralising antibodies did not correlate with spike-specific antibody responses or T-cell responses following boost vaccination [12].

To circumvent the issue of antivector immunity less-prevalent adenoviruses, nonhuman adenoviruses or chimeric adenoviruses that express modifications to the hexon major capsid protein have been increasingly used over recent years [9,70–72]. Higher dosing regimens can also be used to overcome pre-existing vector immunity in the population, but when used with Ad5-nCoV, these higher doses caused increased reactogenicity with limited benefit in immunogenicity [3].

**Prime-boost regimens**

The use of heterologous prime-boost viral vector regimens may overcome the development of antivector immunity and be more immunogenic than homologous regimens [21,73–76]. The use of Ad26 encoding the GP of the Zaire strain of Ebola, followed by an MVA boost, was shown to provide 100% protection against lethal
Ebola when administered to nonhuman primates [77]. This heterologous prime-boost regimen has now been shown to induce strong and durable immune responses in human trials persisting for at least 1 year in both endemic and nonendemic populations [26,27].

Combining viral vectors takes advantage of the differential ability of vectors to prime or boost immune responses. For example, adenoviruses have been shown to prime effective and durable potent B- and T-cell responses, and MVA is able to significantly boost immune responses, but elicits limited humoral responses as a prime [78,79]. However, recent transcriptional data show that an Ad vector boost on an MVA prime appears to augment the molecular response compared with an MVA boost on an Ad prime, including stimulation of preferential TLRs and increased IFN-γ signalling [36], suggesting that further exploration of this area is needed for future vaccine development.

Heterologous prime-boost vaccine schedules using different vaccine platforms have also been evaluated. For example, in vaccines against SARS-CoV-2, a prime dose of adenoviral vector vaccine has been boosted with an mRNA vaccine, which appears to increase vaccine efficacy and immunogenicity against symptomatic infection compared with homologous Ad vaccination [80–83]. In preclinical studies, an MVA booster following mRNA vaccine enhanced specific T-cell responses against HIV-1 [84].

**Improving immunogenicity**

Various methods have been used to further increase the immunogenicity of Ad vectors by enhancing transgene expression and boosting cellular responses. These include the use of endogenous promoters, co-expression of immune-stimulatory molecules and genetic-fusion adjuvants [85,86]. Rollier et al added the Toll-like receptor signalling molecule, TRIF-related adaptor molecule (TRAM), to an adenovirus-based vaccine, showing that co-expression of TRAM and antigen increased the transgene specific CD8+ T-cell responses in mice, but this did not translate into studies in primates [87].

A further way to enhance immunogenicity of viral vectors is to increase immunogen production from vaccine vector. Self-replication via replication-competent vectors allows significant antigen production and may be necessary to induce immunity against some pathogens. The safe use of a replication-competent VSV vector against Ebola virus, VSV-EBOV, in HIV patients, showed that such vectors can be used in immunocompromised patients [88]. An alternative is the use of single-cycle virus vectors, which allow the virus to self-amplify in one additional round of genome replication, circumvent this issue and represent a potential therapy for future viral vector vaccines [89,90].

Harnessing the specific tropism of certain viruses to deliver antigens to desired cell types is a further potential mechanism of improving immunogenicity against certain pathogens. For example, Viktorova et al used a recombinant Newcastle virus, a virus with mucosal tropism, to express proteins from poliovirus, which stimulated systemic and mucosal responses [91].

**Safety**

Adenoviral vector vaccines have now been given to billions of people worldwide. Two vaccines (Ad26.COV2.S and ChadOx1-nCoV-19) have been associated with a very rare clotting disorder, thrombosis with thrombocytopenia syndrome (TTS). This syndrome is characterised by the presence of antiplatelet factor-4 antibodies, although the risk factors for developing TTS and the exact pathogenesis remain unclear [92]. There may be an underlying geographical or genetic link, given variations in the rates of TTS across different populations [93].

**Conclusion and future directions**

Over the past two years, viral vector vaccines have been used as a cornerstone of the control of SARS-CoV-2 in the pandemic, particularly in low- and middle-income countries. The application of newer techniques such as bioinformatics and systems’ serology during this time has provided extensive knowledge on the immunogenicity of the adenoviral vaccine platform.

Although significant advances have been made, further understanding of the spectrum of immune responses stimulated by adenoviral vectors is still needed. Understanding of the mechanism underlying antivector immunity, particularly following repeated dosing, will be vital going forward as vaccines against multiple different pathogens are developed using the same vectors. Evaluating the long-term duration of humoral and cellular responses following widespread Ad vector administration for SARS-CoV-2, and their relationship to vaccine efficacy, will be important in providing invaluable insights into the persistence of immune responses afforded by these vaccine vectors.

Despite the excellent immunogenicity and efficacy of the approved viral vector vaccines, there remains scope to improve immunogenicity. The use of genetic or molecular adjuvants may be a useful strategy, particularly in vaccine vectors that only induce weak or short transgene expression. In addition, the use of heterologous prime-boost regimens, by combining either different viral vectors or different technologies such as mRNA vaccines, has been shown to improve immunogenicity of
homologous regimens, and is likely to play an important role in viral vector vaccine regimens going forward. The use of mucosal viral vector vaccines to induce site-specific immune responses may significantly improve protection, particularly against mucosal pathogens, and clinical trial data from such vaccines are eagerly awaited.

Viral vector vaccines have been a major component of the successful response to the SARS-CoV-2 pandemic. Given their safety, immunogenicity and ability to be modified and scaled up at pace, they will remain an important technology for infectious-disease control in the future.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

**Conflict of interest statement**

AJP is chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation, but does not participate in policy advice on coronavirus vaccines, and is a member of the WHO Strategic Advisory Group of Experts. AJP is a National Institute for Health Research Senior Investigator. TL is named as an inventor on a patent application covering ChAdOx1-nCoV-19. Oxford University has entered into a partnership with AstraZeneca for further development of ChAdOx1-nCoV-19. All other authors declare no competing interests.

**Supporting information**

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.coi.2022.102210.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as: • of special interest ☢ of outstanding interest

1. Smith GL, MacKett M, Moss B: Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. Nature 1983, 302:490-495.

2. Moss B, Smith GL, Gerin JL, Purcell RH: Live recombinant vaccinia virus protects chimpanzees against hepatitis B. Nature 1984, 311:67-69.

3. Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, et al.: Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020, 395:1845-1854.

4. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH, et al.: Immunogenicity and safety of a recombinant adenovirus type-5 vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2020, 396:479-488.

5. Wu L, Zhang Z, Gao H, Li Y, Hou L, Yao H, et al.: Open-label phase I clinical trial of Ad5-EBOV in Africans in China. Hum Vaccines Immunother 2017, 13:2078-2085.

6. 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. Lancet Glob Heal 2017, 5:e324-e334.

7. 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 2015, 389:621-628.

8. Stephenson KE, Le Gars M, Sadoff J, De Groot AM, Heerwegh D, Truayers C, et al.: Immunogenicity of the Ad26.COV2S vaccine for COVID-19. JAMA J Am Med Assoc 2021, 325:1535-1544.

9. Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truayers C, de Groot AM, et al.: Interim results of a phase 1-2a Trial of Ad26.COV2.S Covid-19 vaccine. N Engl J Med 2021, 384:1824-1835.

10. Tukhvatulin AI, Dolzhikova IV, Shchelbyeavov DV, Zhubkova OV, Dzharruulilaaeva AS, Kovskyhina AV, et al.: An open, non-randomised, phase 1/2 trial on the safety, tolerability, and immunogenicity of single-dose vaccine "Sputnik Light" for prevention of coronavirus infection in healthy adults. Lancet Reg Heal Eur 2021, 11:100241.

11. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al.: Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet 2020, 396:467-478.

12. Ramasamy MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, et al.: Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regime in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. Lancet 2020, 396:1979-1993.

This study showed ChAdOx1-nCoV-19 induced similar immune response including anti-spike and anti-RBD antibodies in all age groups including older adults. This was an important finding given the risk of more severe disease with older age and other adenoviral vaccines suggesting reduced immunogenicity in this age group and led to recommendation by Medicines and Healthcare products Regulatory Agency for licensing in UK.

13. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al.: Safety and efficacy of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021, 397:99-111.

14. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al.: Efficacy and effectiveness of an rVSV-vectorized vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet 2015, 389:PS05-P518.

15. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al.: Efficacy and effectiveness of an rVSV-vectorized vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet 2017, 389:P587-P586.

16. Torresi J, McCarthy K, Forderi E, Méric C: Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: randomised controlled phase 3 trials. Vaccine 2010, 28:7993-8000.

17. Monath TP, Guirakhoo F, Nichols R, Yoksan S, Schrader R, Murphy C, et al.: Chimeric live, attenuated vaccine against Japanese Encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese Encephalitis antigen. J Infect Dis 2003, 188:1213-1230.

18. Nasveld PE, Ebringer A, Elmnes N, Bennett S, Yoksan S, Aaskov J, et al.: Long term immunity to live attenuated Japanese encephalitis chimeric virus vaccine: randomized, double-blind,
Vaccines

31. Chéneau C, Eichholz K, Tran TH, Tran TTP, Paris O, Henriquet C, et al.: Efficacy of a tetradivalent dengue vaccine in children in Latin America. *N Engl J Med* 2015, 372:113-123.

32. Logunov DY, Dolzhikova IV, Shcheblyakov DV, Tukhvatullin AI, Shcheblyakov NM, et al.: Safety and immunogenicity of a 2-dose heterologous Ad26.ZEOBV and MVA-BN-Filo Ebola vaccine: a randomized clinical trial. *JAMA J Am Med Assoc* 2016, 315:1610-1623.

33. Mutua G, Anzala O, Luhn K, Robinson C, Bockstal V, Anumendem D, et al.: Safety and immunogenicity of a 2-dose heterologous vaccine regimen with Ad26.ZEOBV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. *J Infect Dis* (1) 2019, 220:57-67.

34. Pollard AJ, Launay O, Liievere JD, Lacabaratz C, Grande S, Goldstein N, et al.: Safety and immunogenicity of a two-dose heterologous Ad26.ZEOBV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2021, 21:493-506.

35. Isola D, Manno D, Afolabi MO, Keshiro B, Bockstal V, Rogers B, et al.: Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEOBV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. *Lancet Infect Dis* 2021, 22:87-109.

36. Iacobelli-Martinez M, Nemerow GR: Preferential activation of toll-like receptor nine by C46-utilizing adenoviruses. *J Virol* 2007, 81:1305-1312.

37. Appleford DM, Patial S, McBride A, Godbehere S, van Rooijen N, Parameswaran N, et al.: Adenovirus vector-induced innate inflammatory mediators, MAPK signaling, as well as adaptive immune responses are dependent upon both TLR2 and TLR9 in vivo. *J Immunol* 2008, 181:2134-2144.

38. Châneau C, Eichholz K, Tran TH, Tran TTP, Paris O, Henriquet C, et al.: Lactoferrin retargets human adenoviruses to TLR4 to induce an abortive NLPR3-associated pyroptotic response in human phagocytes. *Front Immunol* 2021, 12:685218.

39. Rhee EG, Blattman JN, Kasturi SP, Kelley RP, Kaufman DR, Lynch DM, et al.: Multiple innate immune pathways contribute to the immunogenicity of recombinant adenovirus vaccine vectors. *J Virol* 2011, 85:315-323.

40. Coughlan L: Factors which contribute to the immunogenicity of non-replicating adenoviral vectored vaccines. *Front Immunol* 2020, 11:909.

41. Hensley SE, Cun AS, Giles-Davis W, Li Y, Xiang Z, Lasaro MO, et al.: Type I interferon inhibits antibody responses induced by a chimpanzee adenovirus vector. *Mol Ther* 2007, 15:393-403.

42. Quinn KM, Zak DE, Costa A, Yamamoto A, Kastenmuller K, Hill BJ, et al.: Antigen expression determines adenoviral vaccine potency independent of IFN and STING signaling. *J Clin Invest* 2015, 125:1129-1146.

43. Sheerin D, Dold C, O’Connor D, Pollard AJ, Rollier CS: Distinct patterns of whole blood transcriptional responses are induced in mice following immunisation with adeno-associated and poxvirus vector vaccines encoding the same antigen. *BMC Genom* 2021, 22:1-12.[Internet] [Dec 1] [cited 2022 Feb 16] (Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-021-08061-8).

The authors use RNA sequencing to establish that different viral vector vaccines induce distinct transcriptional responses and that the sequence of prime-boost vaccines influenced the magnitude of gene expression changes.

44. Collignon C, Bol V, Chalon A, Surendran N, Morel S, van den Berg RA, et al.: Innate immune responses to chimpanzee adenovirus vector 1555 vaccination in mice and monkeys. *Front Immunol* 2020, 11 (Nov 30).

Authors evaluated cytokine responses and gene expression changes following ChAd1555 vaccination in mice, showing a bidomal pattern of innate changes characterised by IFN-associated signatures.

45. Ewer KJ, O’Hara GA, Duncan CJA, Collins KA, Sheehy SH, Reyes-Sandoval A, et al.: Protective CD8 + T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat Commun* 2013, 4:2836.
The authors used a systems serology approach to characterise vaccine-induced antibodies in humans. They demonstrated that ChAdOx1 booster dose enhanced Fc-mediated functional antibody responses and anti-spike neutralising antibody titres.

51. Planas D, Saunders N, Maes P, et al.: Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 2022, 602:671-675, https://doi.org/10.1038/s41586-022-04389-z https://www.nature.com/articles/s41586-022-04367-2.

52. Dejnirattisai W, et al.: Safety and immunogenicity of a highly attenuated rSIVV4CT1-EOBOVGP1 Ebola virus vaccine: a randomised, double-blind, placebo-controlled trial in healthy adults. J Infect Dis 2019, 220:1127-1135.

53. Clarke DK, Xu R, Matassov D, Latham TE, Ota-Setlik A, Gerardi CS, et al.: Safety and immunogenicity of a highly attenuated rSIVV4CT1-EOBOVGP1 Ebola virus vaccine: a randomised, double-blind, placebo-controlled study in healthy adults. J Infect Dis 2020, 20:455-466.

54. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al.: Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat Med 2021, 27:2032-2040.

55. Sadoff J, Gray G, Vandenbosh A, Čádrans V, Shukarev G, Grinsztejn B, et al.: Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med 2021, 384:2187-2201.

56. Halperin SA, Ye L, MacKinnon-Cameron D, Smith B, Cahn PE, Ruiz-Palacios GM, et al.: Final efficacy analysis, interim safety analysis, and immunogenicity of a single dose of recombinant novel coronavirus vaccine (adenovirus type 5 vector) in adults 18 years and older: an international, multicentre, randomised, double-blind, placebo-controlled phase 3 clinical trial. Lancet Infect Dis 2020, 20:237-248 [Internet] (Jan 15) [cited 2022 Apr 5] (Available from:) (http://www.thelancet.com/article/PIIS14733099(20)307537/fulltext).

57. Barrett JR, Belil-Rammerstorfer S, Dold C, Ewer KJ, Folgatti PM, et al.: Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. Nat Med 2020, 26:279-288 (272) [Internet] (2020 Dec 17) [cited 2022 Jan 27] (Available from:) (https://www.nature.com/articles/s41591-020-01179-4).

58. Mercado NB, Zahn R, Wegmann F, Loos C, Chandrashekar A, Yu J, et al.: Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature 2020, 586:580-588.
Vaccines

75. Casimiro DR, Bett AJ, Fu T, Davies M-E, Tang A, Wilson KA, et al.: Heterologous human immunodeficiency virus Type 1 priming-boosting immunization strategies involving replication-defective adenovirus and poxvirus vaccine vectors. J Virol 2004, 78:11434-11438.

76. Venkatraman N, Ndiaye BP, Bowyer G, Wade D, Sridhar S, Wright et al.: Safety and immunogenicity of a heterologous prime-boost ebola virus vaccine regimen in healthy adults in the United Kingdom and Senegal. J Infect Dis 2019, 219:1187-1197.

77. Callandret B, Vellinga J, Wunderlich K, Rodriguez A, Steigerwald R, Dirmeier U, et al.: A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebola virus and Marburgvirus species in non-human primates. PLoS One 2018, 13:e0192312.

78. 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, 20:1126-1129.

79. Geiben-Lynn R, Greenland JR, Frimpong-Boateng K, Letvin NL: Kinetics of recombinant adenovirus type 5, vaccinia virus, modified vaccinia ankara virus, and DNA antigen expression in vivo and the induction of memory T-lymphocyte responses. Clin Vaccine Immunol 2008, 15:691-696.

80. Liu X, Shaw RH, Stuart ASV, Greenland M, Aley PK, Andrews NJ, et al.: Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vector and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. Lancet 2021, 398:P555-P569.

81. Stuart ASV, Shaw RH, Liu X, Greenland M, Aley PK, Andrews NJ, et al.: Immunogenicity, safety, and reactogenicity of heterologous COVID-19 primary vaccination incorporating mRNA, viral-vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-blind, randomised, phase 2, non-inferiority trial. Lancet 2021, 399:P36-P49.

82. Borobia AM, Carcas AJ, Pérez-Olmeda M, Castaño L, Bertran MJ, García-Pérez J, et al.: Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacc): a multicentre, open-label, randomised, controlled, phase 2 trial. Lancet 2021, 398:P121-P130.

83. Spencer AJ, McKay PF, Beli-Jämmersstorfer S, Ulaszewksa M, Bissett CD, Hu K, et al.: Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. Nat Commun 2021, 12:2893.

84. Gómez CE, Perdiguero B, Usero L, Marcos-Villar L, Miralles L, Leal L, et al., Sorzano COS, Sánchez-Corzo C, Plana M, García F, Esteban M: Enhancement of the HIV-1-Specific Immune Response Induced by an mRNA Vaccine through Boosting with a Poxvirus MVA Vector Expressing the Same Antigen. Vaccines 2021, 9:959, https://doi.org/10.3390/vaccines9090959.

85. Neukirch L, Fougéroux C, Andersson AMC, Holst PJ: The potential of adenoviral vaccine vectors with altered antigen presentation capabilities. Expert Rev Vaccines 2020, 19:25-41.

86. Matchett WE, Malewana GBR, Mudrick H, Medlyn MJ, Barry MA: Genetic Adjuvants in Replicating Single-Cycle Adenovirus Vectors Amplify Systemic and Mucosal Immune Responses against HIV-1 Envelope. Vaccines 2020, 8:64, https://doi.org/10.3390/vaccines8010064.

87. Rollier CS, Spencer AJ, Sogaard KC, et al.: Modification of Adenovirus vector-induced immune responses by expression of a signalling molecule. Sci Rep 2020, 10:5716, https://doi.org/10.1038/s41598-020-61730-8.

Authors added the Toll-like receptors signalling molecule, TRAM, to an Ad vaccine which, when co-expressed with the antigen, increased trangene-specific CD8+ T cell responses in mice.

88. Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al.: Phase 2 Placebo-controlled trial of two vaccines to prevent Ebola in Liberia. N Engl J Med 2017, 377:1438-1447.

89. Crosby CM, Nehete P, Sastry KJ, Barry MA: Amplified and persistent immune responses generated by single-cycle replicating adenovirus vaccines. J Virol 2015, 89:669-675.

90. Anguiano-Zarate SS, Matchett WE, Nehete PN, Sastry JK, Marzi A, Barry MA: A replicating single-cycle adenovirus vaccine against Ebola virus. J Infect Dis 2018, 218:1883-1889.

91. Viktorova EG, Khattar SK, Kouviaskiaa D, Laassri M, Zagorodnyaya T, Dragunsky E, et al.: Newcastle disease virus-based vectored vaccine against poliomyelitis. J Virol 2018, 92:e00976-18.

92. Baker AT, Boyd RJ, Sarkar D, Teijeira-Crespo A, Chan CK, Bates E, et al.: ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome. Sci Adv 2021, 7:8213 [Internet] [Dec 3] [cited 2021 Dec 16] (Available from: ) (https://www.science.org/doi/abs/10.1126/sciadv.abi8213).

93. Soboleva K, Shankar NK, Yadavalli M, Ferreira C, Foskett N, Putsepp K, et al.: Geographical distribution of TTS cases following AZD1222 (ChAdOx1 nCoV-19) vaccination. Lancet Glob Health 2022, 10:e33[Internet] [Jan 1] [cited 2022 Apr 11] (Available from: /pmc/articles/PMC8670752/).