Novel Rabies Vaccines

Hildegund C. J. Ertl

Abstract

Novel rabies vaccines that are less expensive and more immunogenic than current vaccines are needed to reduce the human death toll of rabies. Such vaccines would also allow for more widespread use of rabies vaccines in childhood immunization programs. A number of adjuvants that would allow for dose sparing of current vaccines as well as alternative vaccine prototypes including protein vaccines, genetically modified rabies viruses, pseudotyped viruses, and different types of genetic vaccines are being explored pre-clinically. Some of those have reached early clinical testing. This chapter describes the potential of these different rabies vaccines for use in pre- or post-exposure vaccination.

Introduction

Rabies caused by lyssaviruses, which are divided into three phylogroups and 7 genotypes, claims more than 55,000 human lives annually [1]. Most cases are caused by rabies virus, a phylogroup1 lyssavirus, that forms the basis of current rabies vaccines. About 40% of the deaths occur in children below the age of 15 years. Rabies virus is most commonly transmitted by dogs although other mammals such as cats, raccoons, skunks, foxes, wolves, bats, and others can transmit the virus. In North America, exposure to rabid bats is the main cause for human rabies. Mandatory immunization of dogs has dramatically reduced the incidence of rabies throughout the Americas and Europe but has not yet been successful in Asia and Africa where free-ranging dogs are common. Oral rabies vaccination of wildlife

H. C. J. Ertl (✉)
The Wistar Institute, Philadelphia, PA, USA
e-mail: ertl@wistar.org

© Springer Nature Switzerland AG 2020
H. C. J. Ertl (ed.), *Rabies and Rabies Vaccines*,
https://doi.org/10.1007/978-3-030-21084-7_9

has reduced human rabies virus exposures in Western and Central Europe but is more challenging in the less densely populated areas of the Americas and elsewhere.

Safe and efficacious rabies vaccines for humans are available. They are based on rabies virus that is grown in tissue culture or embryonated eggs and then inactivated. As incubation times for rabies are relatively long and exposures are noteworthy, as they are linked primarily to bites from a rabid animal, vaccines are most commonly given after exposure. Rabies vaccines are given prophylactically to humans at high risk such as veterinarians, wildlife researchers, cavers, or individuals working with the virus. Recently, Peru opted to include rabies pre-exposure prophylaxis (PrEP) into childhood immunization programs in highly endemic remote areas to reduce the increasing death rates due to exposure of children to rabid vampire bats [2, 3]. Recently, the Philippines implemented a similar national program [3, 4].

Currently, licensed rabies vaccines are relatively poorly immunogenic and are thus given repeatedly to achieve adequate titers of neutralizing antibodies, the correlate of protection against infection. For PrEP, rabies vaccines are given three times to reliably induce virus-neutralizing antibody titers ≥ 0.5 international units (IU). Although rabies vaccines induce long-lasting memory B cell responses [5–7], antibody titers eventually wane, necessitating periodic booster immunizations. For post-exposure prophylaxis (PEP), rabies vaccines are given 3–4 times starting as soon as possible after exposure once the wound has been thoroughly cleaned. In case of severe exposure, defined as exposures through transdermal bites or scratches and mucosal contact with saliva or contact with bats, the vaccine is combined with rabies immune globulin (RIG), which should be infiltrated directly to the site of the bite. Rabies vaccines and RIG are expansive and therefore underutilized, which together with lingering ignorance on modes of rabies virus transmission and limited access to health care, results in the high rabies-related human death rates.

More immunogenic vaccines, that achieve virus-neutralizing antibody titers after a single dose and that are less costly, would be expected to increase public access to PrEP and PEP and thereby reduce human death due to rabies. Currently, cost can be reduced by using ID rather than IM immunization. Vaccine injected ID has ready access to the rich network of dendritic cells within the skin [8], which are scarcer within muscle tissue. ID immunization, therefore, triggers more potent immune responses and allows for a reduction of the vaccine dose. Current rabies vaccines given ID at a 5- to 10-fold lower dose compared to the IM dose induce adequate antibody titers. Another way to reduce the cost for rabies vaccinations and increase compliance with recommended regimens is to reduce the number of vaccine doses and tighten immunization schedules from several weeks to one week (https://www.who.int/rabies/PEP_Prophylaxis_guideline_15_12_2014.pdf).

Thus far these changes in rabies vaccination, which are now recommended by WHO and which already prior to that were used extensively in many rabies-endemic countries, have not reduced the efficacy of the rabies vaccine.

This chapter discusses the pros and cons of novel human rabies vaccines under development.

Vaccine-Induced Immune Correlates of Protections

Protection against infection or spread of rabies virus is mediated by neutralizing antibodies that cross-react among viral species within lyssavirus phylogroup 1 [9, 10]. Antibodies induced by current vaccines fail to neutralize phylogroup 2 and other genetically disparate lyssaviruses [11, 12]. Neutralizing antibodies are directed against the lyssavirus glycoprotein, the only protein expressed on the surface of the bullet-shaped virions. The viral glycoprotein forms trimers and most of the neutralizing antibodies are directed against conformation-dependent epitopes. Thus, rabies vaccines must express the viral glycoprotein in its native conformation to induce neutralizing antibodies [11]. Memory B cell responses to current rabies vaccines are long lived and can be recalled decades after the initial immunization [13]. Vaccinated individuals that are exposed to rabies and related lyssaviruses therefore only require two booster immunizations without RIG rather than the full PEP regimen. Induction of B cell responses to the rabies vaccine requires help from $CD4^+$ T cells [14]. Vaccination of individuals with T cell immunodeficiency such as patients suffering from AIDS may thus not result in adequate antibody titers [15]. Most of the antigens of rabies virus, including the glycoprotein, carry T helper cell epitopes [16]. Induction of $CD4^+$ T cells and B cells necessitates stimulation of an initial innate response to drive activation of antigen-presenting cells. Currently licensed rabies vaccines do not contain an adjuvant and rely on intrinsic viral factors to induce inflammatory reactions, such as the single-stranded RNA genome that activates Toll-like receptors 7/8 [17] and double-stranded loops that can activate RIG-I helicase [18].

Requirements for Next-Generation Rabies Vaccines

Novel rabies vaccines need to equal current vaccines in safety and efficacy and surpass their immunogenicity to allow for single-dose regimens and an overall cost reduction. They need to induce innate immunity, which can be achieved with adjuvants. Adjuvants affect the flavor of immune responses by driving activation of type 1 or 2 T helper (Th) cells. Both promote stimulation and affinity maturation of B cells but they achieve different types of class switching. Th1 cells in humans promote stimulation of IgG1 and IgG3 while Th2 responses favor switching to IgG4 and IgA. Although it is currently unknown which antibody isotype is best suited to protect humans against rabies, some evidence obtained in mice suggests that antibodies with Th1-linked isotypes can more readily be induced within the CNS, where they may promote virus clearance [19]. Novel vaccines must express the rabies virus glycoprotein in its native form and they need to induce an antibody response that broadly neutralizes all phylogroup 1 lyssaviruses. Neutralization of phylogroup 2 lyssaviruses or other recently isolated lyssaviruses that belong to neither phylogroups 1 nor 2 [20] would be desirable. Vaccines should induce sustained antibody and memory B cell responses. Vaccines used for PEP must induce an antibody response rapidly before the virus spreads into the nervous

system. As less costly rabies vaccines are most direly needed in developing countries, the vaccine must be heat-stable as cold chains are expensive and difficult to maintain. Delivery needs to be easy.

Several vaccines and vaccine adjuvants have undergone pre-clinical and in part clinical testing. Some meet the criteria required for vaccines use for PEP and PrEP, while others are only suited for PrEP.

Adjuvants

Adjuvants increase the immunogenicity and potency of vaccines by stabilizing the antigen and/or affecting its prolonged release, targeting specific cells or promoting inflammatory responses. Adjuvants that have the latter effect by their very nature increase the reactogenicity of vaccines and on rare occasions even result in serious adverse events as was shown with adjuvanted influenza vaccines [21]. Although tremendous research efforts have focused on the development of new adjuvants very few have reached licensure. Aluminum salts in the form of aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), or mixed aluminum salts have been approved in the United States for more than six decades. Two other adjuvants, i.e., AS04 a combination of aluminum hydroxide and monophosphoryl lipid A, are approved for use with Cervarix, a virus-like particle (VLP) vaccine against oncogenic types of human papillomaviruses [22] and AS03, an oil-in-water emulsion, for H5N1 that is currently not used in the United States in humans but is being stockpiled in case of an avian influenza outbreak [23]. AS03 has been used outside the United States in 47 countries together with the pandemic H1N1 vaccine, where it was linked to increases in narcolepsy in children [24]. No other adjuvants have been licensed in the United States thus far, although many have undergone pre-clinical and early clinical testing. Adjuvants that have been tested in animals with inactivated rabies vaccines are listed in Table 1. Most were tested in mice, where they increased antibody responses to the rabies vaccine and thus protection against challenge. The caveat should be pointed out that results obtained in rodents, especially with adjuvants that target pathogen recognition receptors [37], may not necessarily translate to humans due to differences in innate receptor specificity and distribution [38].

Data obtained in humans and nonhuman primates indicate that an alum-adjuvanted rabies vaccine would provide limited advantages [25]. Two adjuvants, ISCOMATRIX™, a particle forming adjuvant composed of cholesterol, phospholipid, and saponin and IMO-2170, an immune modulatory oligonucleotide with agonist activity to TLR-9, yielded promising results in nonhuman primates [30]. One adjuvant, a TLR-3 agonist based on a synthetic dsRNA analogue and a refined form of polyinosinic-polycytidylic acid stabilized with kanamycin and calcium has been tested in human volunteers with no prior history of rabies exposure or vaccination [36]. Group 1 received RABIPUR in a 4-dose i.m. regimen (1-1-1-1), group 2 received the same regimen with the adjuvanted vaccine called PIKA® rabies vaccine, group 3 received the PIKA® rabies vaccine in an accelerated 3 visit regimen

Table 1 Adjuvants tested with conventional rabies vaccines

Adjuvant	Vaccine	Species	Study outcome	References
Aluminum hydroxide	HDCSV + adjuvant given s.c. compared to HDCSV, i.m., single or 8-site i.d. regimen	Humans	Response to vaccine with adjuvant equal to single-site i.d. regimen without adjuvant, inferior to 8-site i.d. regimen	[25]
IL-2, given daily systemically	Attenuated SAD strain of rabies	Outbred mice	Enhanced vaccine potency tested for by challenge with CVS-11 virus	[26]
Polar glycopeptidolipids from *Mycobacterium chelonae*	Semple vaccine	BALB/c mice	Enhanced vaccine potency tested for by challenge with CVS virus	[27]
CpG ODN (BW006)	Inactivated rabies virus strain CTN	BALB/c mice	Increased titer of neutralizing antibodies, increased protection upon challenge	[28]
Activation-associated protein-1 from *Onchocerca volvulus*	VERORAB	BALB/c mice	Increased IgG1 and IgG2a antibody responses to the rabies vaccine	[29]
ISCOMATRIX™ adjuvant	Rabavert®	Rhesus macaques	Significantly increased titers of neutralizing antibodies	[30]
IMO-2170, synthetic TLR9 agonist	Rabavert®	Rhesus macaques	Significantly increased titers of neutralizing antibodies	
Amorphous aluminum hydroxylphosphate sulfate	Rabavert®	Rhesus macaques	Marginally increased titers of neutralizing antibodies	
Uridine 5′-triphosphate	Commercial rabies vaccine (single dose)	BALB/c mice	Marginally increased protection in PrEP compared to a suboptimal dose of vaccine	[31]
Ginsenoside Re (Re) is a saponin from Panax ginseng	Rabvac®	Outbred mice	Enhanced and prolonged antibody responses	[32]
Salmonella typhimurium flagellin	Whole-killed rabies vaccines	BALB/c mice	Slightly increased antibody responses	[33]

(continued)

Table 1 (continued)

Adjuvant	Vaccine	Species	Study outcome	References
Polysaccharides IIP-A-1 and IIP-2 from *Isatis indigotica* root	Whole-killed rabies vaccines	BALB/c mice	Accelerated and increased antibody responses	[34]
Hydrogenated soya phosphatide and cholesterol liposomes	Whole-killed rabies vaccines	BALB/c mice	Slightly increased vaccine potency	[35]
TLR-3 agonist	RABIPUR®	Humans	Well tolerated, increased immunogenicity of the vaccine	[36]

(2-2-1). The experimental vaccine was well-tolerated although 1/12 subjects of group 2 had to be taken off the trial due to pruritus that eventually resolved. Group 3 developed titers of rabies neutralizing antibodies faster than control group 1 and antibody responses were more sustained. Group 2 showed a trend toward accelerated and prolonged responses, which failed to reach significance. Although the results of phase I safety trial were promising, questions on improved immunogenicity remain. They will need to be addressed in a larger phase II trial. Specifically, the most promising accelerated 2-2-1 regimen for the PIKA® rabies vaccine was compared to the standard 1-1-1-1 regimen, which opens the question if the faster and enhanced response was indeed caused by the adjuvant or differences in dosing and timing of vaccination.

Protein and Peptide Vaccines

Protein or peptide vaccines are based on the viral glycoprotein, a 65-k Da protein that contains an intracytoplasmic domain, a hydrophobic transmembrane domain, and an ectodomain. After synthesis, the protein forms trimers and is N-glycosylated minimally at one of three potential sites, before it is transported to the cell surface [24]. Protein vaccines based on the rabies virus glycoprotein have the advantage that they are exceptionally safe and they can be used for both PEP and PrEP. Depending on the source of the protein, such as plant cells, they may also be cost effective. Disadvantages of protein vaccines are that correct folding into the native trimeric structure of the rabies virus glycoprotein, which is essential for the induction of neutralizing antibodies, remains a challenge. Full-length glycoprotein is poorly soluble due to the high hydrophobicity of the transmembrane domain [39], which is nevertheless essential for the protein's correct folding.

Different types of protein and peptide vaccines that have been explored are listed in Table 2. Rabies virus glycoprotein produced in mammalian cells, such human embryonic kidney (HEK) 293 cells [46], baby hamster kidney (BHK)-21 cells [40],

Table 2 Protein and peptide vaccines to rabies virus

Expression system	Protein	Immunization protocol	Species	Results	References
Protein vaccines					
Mammalian expression systems					
HEK-293 cells	VLP-forming glycoprotein	Two immunizations	Mice	Protection	[40]
BH-K21 cells	Glycoprotein	No immunization studies			[41]
NA cells	Glycoprotein	No immunization studies			[41]
Insect cell expression systems					
Baculovirus in Sf9 cells	Purified glycoprotein + alum	2 doses, 200–400 ng	Mice	Protection	[36]
	Unpurified glycoprotein	~12–120 μg	Mice, Racoons	Protection	[30, 42]
	Glycoprotein nanoparticles	1–5 μg	Mice	Superior immunogenicity to Rabipur	[43]
		2–3 doses, 5–50 mg	Humans	Results unknown	[44, 45]
Drosophila melanogaster Schneider 2 cells	Glycoprotein	No immunization studies			[46]
	Purified glycoprotein	3 μg of purified protein	Mice	Modest neutralizing antibody response	[39]
Yeast expression systems					
Saccharomyces cerevisiae	Unpurified glycoprotein	Yeast extract	Mice	No protection	[40]
Pichia pastoris	Glycoprotein	No immunization studies			[41]
Pichia angusta	Truncated glycoprotein	No immunization studies			[47]
Plant expression systems					
Maize	Unpurified glycoprotein	1 dose, 50 μg, orally	Mice	Protection	[48]
		1 dose, 0.5–2 mg, orally	Sheep	Partial protection	[49]
Tobacco plants	Purified glycoprotein + Freund's adjuvant	4 doses, 25 μg, i.p.	Mice	Protection	[50]

(continued)

Table 2 (continued)

Expression system	Protein	Immunization protocol	Species	Results	References
Spinach	Tobacco mosaic virus expressing antigenic determinants of rabies virus	3 doses, 50 μg, i.p.	Outbred mice	Partial protection	[51]
		3 doses, spinach leaves, orally	Humans	Induction of primary and recall B cell responses in some individuals	[50, 52]
Carrots	Rabies virus protein in raw carrots	50 μg of glycoprotein + 50 μg of nucleoprotein	Mice	Partial protection	[53]
Peptide vaccines					
Branched lipopeptide vaccine	$CD4^+$ and $CD8^+$ T cell epitopes	3 doses, 50 μg, i.p.	Mice	Induction of T cells	[54]
Lipopeptide vaccine adjuvanted with TLR-7	$CD4^+$ and $CD8^+$ T cell epitopes + TLR-7 agonist	1 dose, 50 μg combined with a rabies vaccine	Mice	Partial protection	[55]
Multi-epitope-based vaccine coated with canine gp69	conserved B cell epitopes coated with canine gp69 and given in an oil in water emulation	1 dose	Mice, dogs	Partial protection	[56]

or neuroblastoma cells [41] is glycosylated, although the glycosylation pattern, which in turn affects the protein's immunogenicity, varies depending on the host cell type. It is furthermore affected by culture conditions. Expression in insect cell systems, such as by baculovirus in *Spodoptera frugiperda* (Sf-9) cells [47, 48] or in *Drosophila melanogaster* Schneider 2 cells [50, 53], also results in oligomeric glycoprotein able to induce virus-neutralizing antibodies in mice. A baculovirus-derived glycoprotein that spontaneously forms micelles (nanoparticles) has undergone testing in mice. A 3-dose regimen induces higher rates of seroconversion than commercial Rabipur vaccine. This vaccine, developed by CBL Biological, has undergone phase I and II trials in humans and a phase III trial is planned. Results of these trials are not yet available to the public.

Expression in *Saccharomyces cerevisiae* [51] resulted in a protein that failed to be immunogenic in mice, which may relate to the typically high mannose glycosylation pattern by yeast. Expression in methylotrophic yeast *Pichia pastoris* was also assessed, resulting in a protein that at least in part was correctly folded [52]. Similar results were obtained in *Pichia angusta* (*Hansenula polymorpha*) [54]. Neither protein was assessed for induction of neutralizing antibodies in animals, the ultimate test for correct folding and glycosylation of the rabies virus glycoprotein.

The rabies virus glycoprotein has been produced in plant cells, including maize [56], *Nicotiana tabacum* plants [57], carrots [58], or spinach [59, 60]. The latter expressed epitopes of the glycoprotein and the nucleoprotein together with the alpha mosaic virus coat protein to facilitate oral vaccine delivery. Plant cells appropriately glycosylate the rabies virus glycoprotein. However, successes upon oral immunization varied. A maize-derived glycoprotein fed at 50 μg/dose within the kernels to mice resulted in protection against challenge with a vampire bat virus [56]. The chimeric spinach-derived peptide vaccine was initially tested after intraperitoneal injection of mice, where it achieved protective immunity after three doses [59]. Humans were fed three times raw spinach containing the chimeric rabies virus peptides [60]. Volunteers without prior immunization with the rabies vaccines were then immunized with one dose of a commercial rabies vaccine. Seven days, later 3 of 9 individuals had developed neutralizing antibodies, indicating that the plant vaccine had primed a B cell response in at least some of the human volunteers. In another arm of the study, humans who had previously been immunized with a commercial rabies vaccine were fed the same material. In this group, 3 of 5 showed a recall response. Another study demonstrated in mice the immunogenicity and efficacy of a purified rabies virus glycoprotein grown in *Nicotiana tabacum* leaves [57] or in carrots [58].

Results obtained with some of the expression systems, especially the virus-like particles (VLPs) formed in HEK 293 cells and the maize-produced glycoprotein, are promising. Unless the glycoprotein is used for oral immunization, it must be purified, which adds another layer of complexity and cost to protein vaccines. Oral immunization with raw material, such as maize kernels, carrots, or spinach leaves, may provide challenges of accurate dosing, not only of the ingested material but also of the amount of antigen that eventually is presented to the immune system. Several groups have explored the use of peptides. A branched lipopeptide vaccine, with or

without TLR7 agonist, was shown to induce T cell responses to rabies virus that could accelerate the B cell response to a traditional vaccine [61]. A multi-epitope-based vaccine coated with canine gp69 tested in mice showed limited efficacy [62]. Considering that rabies vaccines need to induce a very broad antibody response against multiple isolates and preferentially different genotypes, this approach is unlikely to replace current vaccines.

Genetically Altered Rabies Vaccines

Rabies virus can be modified by reverse genetics [63]. This has led to the production of highly attenuated rabies virus and/or virus with improved immunogenicity that could be used for animal or human vaccination (Table 3). Depletion of the P gene, which encodes part of the viral polymerase, attenuates rabies virus. The virus is apathogenic even if injected intracerebrally into adult or suckling immunocompetent mice or into immunodeficient mice, although in the latter the P gene-deleted vaccine spread from the periphery into the central nervous system. The P gene-deleted rabies virus induces a virus-neutralizing antibody response [64]. The response comes up fairly slowing but then surpasses that of an inactivated vaccine based on wild-type virus, suggesting that such a construct may be considered for PReP, but not PEP. To enhance the immunogenicity of the P-gene deleted rabies vaccine, it was further modified to express two copies of the viral glycoprotein gene. This vaccine more rapidly induced rabies virus neutralizing antibodies in mice and nonhuman primates [65]. Rabies virus depleted of the matrix (M) gene is also apathogenic and unlike the P gene-deleted virus does not disseminate into the brain of immunodeficient mice. This vaccine very rapidly induces virus-neutralizing antibodies in mice and a somewhat slower response in nonhuman primates [66]. Both types of viruses induce a Th1-biased response.

Attenuated viral vaccines are attractive, as they produce higher and more sustained levels of antigen than an equal dose of an inactivated virus. Nevertheless, considering that rabies is nearly always fatal, a live vaccine, even if it is shown by all possible means to be safe in animals, may not be accepted by regulatory authorities or the public. Attenuated rabies vaccines could be useful for the treatment of human patients with active symptoms of a CNS rabies virus infection [67].

To overcome this potential safety limitation, mutants, which carry two copies of the glycoprotein gene, were inactivated and then tested in dogs, in comparison to a traditional vaccine [68]. After a single dose, the mutant virus-induced an accelerate antibody response that protected ~80% of the animals against a challenge with a virulent strain of rabies virus. This vaccine could be suitable for PEP where the speed of onset of a virus-neutralizing antibody response is of the essence. A further advantage of the mutant vaccine is its superior growth in BHK-21 cells, compared to the parental HEP-Flury strain, although additional studies are needed to assess if this endures scale-up of vaccine production.

Table 3 Genetically altered rabies vaccines

Rabies virus	Type of vaccine	Immunization protocol	Species	Results	References
P gene-deficient rabies virus	Live attenuated virus	1 dose, 10^6 FFUs, i.p.	Mice	Induction of neutralizing antibodies and protection against challenge, superior to the efficacy of an inactivated wild-type rabies virus	[58]
Modified P gene-deleted rabies virus	Live attenuated virus	1 dose,10^3–10^5 FFUs, i.m.	Mice	Rapid induction of neutralizing antibodies and protection against challenge	[59]
M gene-deficient rabies virus	Live attenuated virus	1 dose, 10^3–10^5 FFU, i.m.	Mice	Rapid induction of neutralizing antibodies, complete protection against challenges	[60]
		2 doses, 6×10^7 FFUs, i.m.	Nonhuman primates	Induction of neutralizing antibodies	
Rabies virus with two glycoprotein gene copies	Inactivated virus	1 dose, equivalent of 10^7 FFU with adjuvant, s.c.	Dogs	Slightly accelerated neutralizing antibody response, 83% protection against challenge	[62]

FFU focus forming unit

Virus Particle Vaccines

Some viruses can be modified to express the rabies virus glycoprotein on the surface of the virion (Table 4). These viruses share the advantage of protein vaccines or genetically modified rabies virus in that the protein is instantly available for induction of immune responses thus potentially allowing for their use in PEP. They furthermore have the advantage that the inflammatory response to the parent virus renders them independent from added adjuvants. Purification methods for viruses are

Table 4 Pseudotyped viruses as vaccines to rabies virus

Pseudotyped virus	Immunization protocol	Species	Results	References
New castle disease virus	1 dose, ~10^6–10^8 egg infective doses, i.m.	Mice	Complete protection	[63]
	3 doses, ~10^8–10^{10} egg infective doses, i.m.	Cats, Dogs		
Baculovirus	2 doses, 10^8 IFU, i.m.	Mice	Complete protection	[64]
Parainfluenza virus 5	1 dose,10^8 PFU, i.m., i.n., oral, PrEP	Mice	Complete protection	[65]
	3 doses, 10^7 PFU, intracerebrally, PEP	Mice	Partial protection	[69]

EID egg infectious unit, IFU infectious unit, PFU plaque-forming unit

well established and not affected by peculiarities of the rabies virus glycoprotein such as the high hydrophobicity of its transmembrane domain. Potential disadvantages are pre-existing immunity to the parent virus or modest levels of the displayed rabies virus glycoprotein, both of which could dampen immune responses to rabies virus. One also needs to consider an increased potential for adverse events due to pathogenicity of the parent virus.

Newcastle disease virus, an avian parainfluenza virus, was modified to express the rabies virus glycoprotein on its surface. The vaccine could be grown to high titers in embryonated chicken eggs and was shown to be safe in mice, dogs, and cats. It induces high and sustained titers of rabies virus-neutralizing antibodies. A single intramuscular dose achieved complete protection in mice, while a triple immunization regimen was shown to protect cats and dogs [70].

A baculovirus was modified to express the rabies virus glycoprotein on its surface and to simultaneously express another glycoprotein under the control of the CMV promoter. This vector thus serves as both a VLP and a genetic vaccine. In mice two, doses of the recombinant baculovirus induce a virus-neutralizing antibody response and complete protection against challenge [71].

Parainfluenza virus 5 vectors expressing the rabies virus glycoprotein were developed and tested initially in a PrEP regimen, where they induced neutralizing antibodies in mice and complete protection against challenge [72]. In the same study, the pseudotyped virus was tested in a PEP regimen in mice. Mice were challenged intramuscularly with rabies virus and then vaccinated three times intracerebrally with the recombinant virus starting 4–6 days after challenge. A significant reduction in clinical signs was observed, although it should be noted that early vaccination with the wild-type virus also reduced illness [72].

Although the pseudotyped virus may be a cost-effective alternative for PEP, additional studies are needed in more relevant animal models.

Genetic Vaccines

Genetic vaccines are vaccines that introduce genetic material or transcripts of a pathogen. Such vaccines need to transduce cells. They use the host cell machinery to produce the immunogen, which then stimulates an immune response. Onset of an immune response to a genetic vaccine is delayed and although this delay may be marginal and synthesis of proteins would be expected to occur within hours leading to steady accumulation of antigen over a span of several days, it nevertheless precludes the use of genetic vaccines for PEP, where speed of the neutralizing antibody response determines whether a patient will live or die.

Genetic vaccines that have been explored for vaccination against rabies virus can be subdivided into mRNA vaccines, DNA vaccines in the form of plasmid vectors or replicons, viral, and bacterial recombinant vaccines (Table 5).

mRNA Vaccines

Pre-clinical studies showed that mRNA encoding the rabies virus glycoprotein injected into animal transduces cells and stimulates a Th2-biased antibody response against rabies virus that protects mice and pigs against challenge [103]. Based on these promising results, a lyophilized mRNA vaccine encoding the glycoprotein of the Pasteur strain of rabies virus, termed CV7201, was tested in a dose escalation trial in human volunteers without previous exposure to a rabies vaccine [104]. The vaccine was given 2 or 3 times intramuscularly on days 0, 28, and 56 or 3 times intradermally on days 0, 7, and 28. Injections were either given by a syringe or an injector device. Ninety percent of participants reported side effects, in 12% of those side effects were considered severe. Local side effects were more common after intradermal application, systemic side effects tended to increase at higher vaccine doses. Intramuscular or intradermal immunization by syringe failed to elicit rabies virus-specific neutralizing antibody titers at or above 0.5 IU/ml. Using injector devises about ~50% of individuals that received the vaccine at the highest dose (400 μg/dose) intramuscularly and ~70% of those injected intradermally developed titers of or above 0.5 IU/ml. By one year after immunization titers in all individuals declined to below 0.5 IU/ml. Some of the individuals who had been primed intradermally with the injector device (80 μg/dose, 3 doses) were boosted one year later with the same vaccine dose, route, and type of injection used for priming. After the boost, ~60% of individuals achieved antibody titers above 0.5 IU/ml. Although this proof of principle studies shows that an mRNA vaccine can induce an immune response in humans, the trial outcome is far from impressive. Considering the severity of rabies, a vaccine that does not achieve adequate titers in all vaccine recipients is not acceptable.

Table 5 Genetic vaccines

Type of vaccine	Immunization protocol	Species	Results	References
mRNA vaccines				
mRNA, PrEP	2–3 times, i.m. or i.d., syringe or injection devise	Humans	Short-lived titers of neutralizing antibodies in some volunteers	[67]
DNA vaccines				
DNA, PrEP	1–2 doses, 0.1–50 μg, i.m. or i.d., syringe or gene gun	Mice	Protective immunity	[73–75]
DNA encoding chimeric glycoprotein, PrEP	1 dose, 5–80 μg, i.m. after cardiotoxin treatment	Mice	Broadly cross-reactive titers of neutralizing antibodies	[76]
DNA, PrEP	2 doses, 100–300 μg, i.m. or i.d.	Dogs, cats	Neutralizing antibodies, protection against challenge	[77]
DNA, PrEP	1 dose, 100–1000 μg, i.m.; 6–60 μg, gene gun	Nonhuman primates	Sustained neutralizing antibody responses, protection against challenge	[78]
DNA + genetic GM-CSF adjuvant, PrEP	1 dose, 1–5 μg, i.m.	Mice	Increased vaccine efficacy	[79]
DNA + monophosphoryl lipid, PrEP	2 doses, 2 μg, various routes	Mice	Increased antibody responses after priming	[80]
DNA + alum, PrEP	1 dose, 100 μg, i.m.	Mice	Increased antibody responses and protection	[81]
DNA + alum or cationic lipid, PrEP	2 doses, 200 μg, i.m.	Horses	Neutralizing antibody responses to DNA vaccine + adjuvant	[82]
DNA + amine-terminated poly (ether imine) dendrimer, PrEP	3 doses, 90 μg, i.m.	Mice	Increased antibody responses and protection	[83]
DNA + Emulsigen-D, PrEP	3 doses 100 μg, i.m.; 1 μg, gene gun	Mice	Increased antibody responses and protection	[84]
DNA prime, adenovirus vector boost, PrEP	1 dose, 50 μg, various routes	Mice	Increase antibody responses in presence of pre-existing neutralizing antibodies to the viral vector	[85]
DNA, PEP	5 doses, 100 μg, i.m. + RIG	Mice	Partial protection	[86]
	1 dose, 100 μg, i.m.			[87]

DNA + Emulsigen-D, PEP	5 doses, 100 μg, i.m.	Mice	Complete protection	[84]
DNA	1 dose, 60 μg, gene gun + RIG	Nonhuman primates	Partial protection	[88]
DNA, PEP	4 doses, 50–100 μg, i.n.	Mice, rabbits	Partial protection	[89]
Replicon vaccines				
Sinbis replicon	1 dose, 50 μg, i.m.	Mice	Complete protection	[90]
Poxvirus vaccines				
Vaccinia virus	1 dose, various amounts and routes	Numerous species	Complete protection	[91–94]
Modified Vaccinia Ankara	1 dose, 10^7–10^9 pfu, i.m.	Mice	Partial protection	[95]
Canarypox virus	1 dose	Cats and dogs	Protection	[96, 97]
Canarypox virus	1 dose, $10^{3.5}$–$10^{5.5}$ $TCID_{50}$, i.m.	Humans	Induction of modest neutralizing antibody responses	[98]
Parapoxvirus	2 doses, 10^8–10^5 $TCID_{50}$ various routes	Mice	Complete protection at the higher doses	[99]
	2 doses, $10^{7.5}$ $TCID_{50}$, i.m. or s.c	Dogs	Rapid onset of neutralizing antibody responses, and enhanced antibody titers at higher doses	[99]
	2 doses 10^5–10^8 $TCID_{50}$, i.m.	Cats	Neutralizing antibody titers	[99]
Adenovirus vectors				
Replication-competent adenovirus (HAdV5)	1 dose, 10^5–10^8 IU, oral	Mice, skunks	Induction of neutralizing antibodies in most animals	[99]
Replication-defective adenovirus (HAdV5)	1 dose, 2×10^4–2×10^6 IU, various routes	Mice	Complete protection upon i.m. immunization	[100]
Replication-defective adenovirus (SAdV25)	1 dose, 5×10^3–5×10^7 IU, s.c. or i.n.	Mice	Complete protection at the higher doses	[101]
Replication-defective adenovirus (SAdV25)	1 dose, 10^9 vp, i.m.	Nonhuman primates	Complete protection	[102]

DNA Vaccines

Plasmid vectors also called DNA vaccines can be given by intramuscular or intradermal injection. They can be applied intradermally upon coating to gold beads with a gene gun. The plasmids transduce cells locally and then produce the antigen [73]. Transduction rates can be increased by electroporation following vector injection [74]. DNA vaccines have clear advantages. They are very easy to produce. They are heat stable. They carry their own adjuvant in the form of CpG sequences within the vector genome, which activate TLR-9 [75]. Their testing in humans has shown that they are well tolerated [76, 78]. They induce a full range of immune responses including Th1-based antibodies. Immune responses tend to be sustained. Their main disadvantage is that their potent immunogenicity in animals has not reliably translated to human studies [77].

Several studies have tested DNA vaccines expressing the rabies virus glycoprotein. Initial studies showed that a single dose of 50 μg of a DNA vaccine given i.m. protected 50% of mice against challenge while 3 doses achieved 80% protection [79]. Subsequent studies reported complete protection after a single i.m. dose of 10 μg of DNA [80] or 2 μg given by gene gun [81]. Using a DNA vaccine expressing a chimeric glycoprotein composed of phylogroup 1 and 2 lyssaviruses or 2 DNA vaccines expressing the entire glycoprotein sequences of these two viruses resulted in a broadly neutralizing antibody response that neutralized most genotypes of lyssavirus [82]. Other studies showed induction of virus-neutralizing antibody responses in nonhuman primates [83], dogs, and cats [84] that as far as was tested protected against challenge. Responses could be increased by adding either genetic adjuvants in form of a second DNA vector expressing a cytokine [85] or by formulating the DNA vaccine in a traditional adjuvants such as monophosphoryl lipid [87], alum [86], cationic lipids [88], amine-terminated poly(ether imine) dendrimer [89], or Emulsigen-D [90]. Alternatively, responses could be enhanced by using a second vaccine such as an adenovirus vector for a booster immunization [105]. In such prime-boost regimens, DNA vaccines were shown to overcome impairment of transgene product-specific B cell responses by pre-existing neutralizing antibodies to the viral vaccine vector [105]. The initial studies focused on PrEP although a number of studies reported reduced mortality by using rabies DNA vaccine in PEP regimens in mice or nonhuman primates [106–109].

Viral replicons such as those based on Sindbis virus replicons were also shown to induce protective levels of neutralizing antibodies in mice and dogs after a single dose [110].

DNA vaccines for other pathogens, such as *Plasmodium falciparum* [91], HIV-1 [92], Hantaan virus [95], Ebola, and Marburg viruses [96] have undergone clinical testing. Immunogenicity was variable. More potent responses were achieved in clinical trials that used DNA vaccines for priming followed by a boost with a viral vector [98]. Such an approach would not simplify current rabies vaccine regimens.

Viral Vector Vaccines

Recombinant viruses, similar to DNA vaccines, induce immune responses after they infect cells in vivo and transcribe the inserted sequence. They have the advantage over DNA vaccines that infection rates are higher which together with more potent signaling to the innate immune system increases their immunogenicity. Their safety profile varies, those that are replication-defective such as E1-deleted adenovirus (Ad) vectors are generally well tolerated [99, 111, 112] while some of the poxvirus vectors are too reactogenic for use in humans [113]. One clear disadvantage of viral vector vaccines is that their immunogenicity is reduced in the presence of pre-existing vector-specific neutralizing antibodies induced by natural infections or previous vaccinations [114, 115]. They are thus suitable for single vaccine regimens but the same vector should not be used for repeated immunizations. Single-cycle flavivirus vectors expressing the rabies virus glycoprotein have been developed. They showed immunogenicity and efficacy against a rabies virus challenge in experimental animals [116]. This vaccine platform has not yet been tested in clinical trials and it is thus impossible to predict its potential for cost-effective scale-up and its performance in humans.

Poxvirus Vectors

Several types of poxviruses have been vectored and used as rabies vaccines (Table 5). Vaccinia virus recombinants expressing the rabies virus glycoprotein are being used for immunization of wildlife [100–102, 117–119]. Although they are highly immunogenic and reliably induce protective immunity after a single dose, their residual virulence precludes their use in humans [120]. Vectors based on Modified Vaccinia Ankara (MVA) are more attenuated. The virus, upon serial passages in cell lines that caused deletions of ~10% of its genome, is no longer capable to replicate in primate cells. This attenuation reduces the vectors' immunogenicity and in mice, an MVA recombinant expressing the rabies virus glycoprotein achieved only partial protection [119]. Other poxviruses, such as canarypox virus, have been vectored to express the rabies virus glycoprotein. The canarypox vaccine showed efficacy in cats [100] and is now licensed for this species as PureVax feline rabies for 1- and 3-year duration of immunity vaccines. The vaccine was also tested in a three-dose regimen in human volunteers in comparison to a traditional tissue culture-derived rabies vaccine. The vaccine was well-tolerated and induced rabies virus-neutralizing antibodies. Titers, which contracted rapidly, were well below those achieved with the commercial vaccine [101]. A recombinant parapoxvirus induced adequate titers of neutralizing antibodies in mice, dogs, and cats but again its immunogenicity was below that of the vaccinia virus recombinant [102].

Poxvirus vectors, although licensed for wildlife immunization and for routine vaccination of cats are overall poor candidates as single-dose vaccines for humans—those that are highly immunogenic are too reactogenic and those that are more attenuated lack immunogenicity.

Adenovirus Vectors

Adenoviruses cause species-specific infections. Multiple serotypes have been isolated from various species and some of those derived from human or simian serotypes have undergone clinical testing as preventative vaccines for a plethora of pathogens [99, 111, 112, 121–123].

Adenovirus vectors can be constructed to retain their replication competence by inserting foreign sequences into the deleted E3 domain that encodes polypeptides that are non-essential for virus replication but serve to subvert immune responses. Replication-competent adenovirus vectors based on human serotype 5 (HAdV5) expressing the rabies virus glycoprotein have been licensed in North America for immunization of wildlife [124]. Due to their potential virulence in their human host where wild-type HAdV5 virus can cause pneumonia, gastroenteritis, and/or hepatitis, such vectors are not suited for use in humans [125].

Adenoviruses are rendered replication defective by insertion of sequences into the deleted E1 domain, which encodes proteins that are essential for the transcription of the other viral genes. E1-deleted adenovirus vectors induce very potent T and B cell responses that, due to low-level persistence of the viral vectors, are exceptionally sustained [126]. They are well tolerated in humans if used at immunogenic doses. High doses elicit severe side effects due to the induction of strong innate immune responses. Production and purification methods for use in humans are well established [127]. Methods to preserve adenovirus vectors independent of cold chains are available [43, 128]. It is likely that adenovirus vector vaccines for rabies virus would be cost effective, as it is estimated that a single-dose vaccine could cost as little as one dollar [44].

The main disadvantage of adenovirus vectors is that their immunogenicity and efficacy are impaired by pre-existing neutralizing antibodies to the vector [114, 115]. Adenoviruses are ubiquitous and most humans become infected early during childhood with different serotypes. The prevalence of neutralizing antibodies depends on the virus serotype and the geographic location. Around 40% of human adults in the United States or Europe are seropositive for HAdV5, the best-studied serotype, while rates exceed 80% in some African counties [44]. Antibodies to serotypes such as HAdV26 are rare in the United States or Europe but again common in Africa [45]. Human serotypes of recombinant adenoviruses expressing the rabies virus glycoprotein, although they have yielded promising results in animal studies [49], are thus not suited for immunization of humans. Vectors based on viruses isolated from nonhuman primates, such as chimpanzees, have been generated. These viruses, which are phylogenetically closely related to human serotypes, do not circulate in the human population [55]. Most human adults thus lack neutralizing antibodies to simian adenoviruses and those that have antibodies tend to have very low titers. An E1-deleted chimpanzee adenovirus SAdV-25 (also called AdC68) expressing the rabies virus glycoprotein has been tested extensively in mice and nonhuman primates [55, 69]. The virus induces, after a single intramuscular dose, potent and sustained virus-neutralizing antibody responses, which can readily be boosted by rabies virus. Animals, including nonhuman primates, were shown to be

completely protected against challenge given more than a year after vaccination. This vaccine, which is scheduled for clinical testing, is thus highly suited for PrEP. It would provide a cost-effective alternative to the current rabies vaccine and would thus allow for more wide-spread incorporation of rabies vaccination into childhood immunization programs. As remains to be tested antibody responses could be increased by a prime-boost regimen with two heterologous chimpanzee adenovirus vectors [93], which may broaden the efficacy of the vaccine to lyssaviruses that do not belong to phylogroup 1.

Summary

The number of rabies vaccines that have undergone pre-clinical testing is impressive but only a few of those have undergone clinical testing where most were shown to be relatively ineffective. The most promising approaches right now are the adjuvanted PIKA rabies vaccine that through the addition of a TLR-3 adjuvant increases the immunogenicity of a licensed vaccine and may thereby allow for dose sparing [36]. One protein vaccine based on glycoprotein VLPs produced in baculovirus is scheduled for phase III clinical trials and although results from the earlier clinical trials have not been published thus far, one would assume that the vaccine has shown safety and immunogenicity and overall non-inferiority to current vaccines in phase I/II trials. Attenuated rabies virus is unlikely to replace current vaccines but genetically modified inactivated rabies vaccines that express two copies of the rabies virus glycoprotein may be useful for PrEP and PEP [68]. Pseudotyped viruses have undergone limited testing, where they gave promising results but concerns about toxicity in humans may hinder their transition toward clinical trials [70–72]. The above-described four types of vaccines could be used in PEP and PrEP unlike genetic vaccines that due to a delayed onset of expression of the immunogen should only be considered for PrEP. The most promising genetic vaccine is the E1-deleted SAdV-25 vector that may be sufficiently cost effective for inclusion into childhood immunization programs in highly rabies endemic areas [69].

References

1. Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, Barrat J, Blanton JD, Briggs DJ, Cleaveland S, Costa P, Freuling CM, Hiby E, Knopf L, Leanes F, Meslin FX, Metlin A, Miranda ME, Müller T, Nel LH, Recuenco S, Rupprecht CE, Schumacher C, Taylor L, Vigilato MA, Zinsstag J, Dushoff J. Global alliance for rabies control partners for rabies prevention. Estimating the global burden of endemic canine rabies. PLoS Negl Trop Dis. 2015;9:e0003709. https://doi.org/10.1371/journal.pntd.0003709.eCollection.
2. Condori-Condori RE, Streicker DG, Cabezas-Sanchez C, Velasco-Villa A. Enzootic and epizootic rabies associated with vampire bats, peru. Emerg Infect Dis. 2013; https://doi.org/10.3201/eid1809.130083.
3. Dodet B. Asian Rabies Expert Bureau (AREB). Report of the sixth AREB meeting, Manila, The Philippines, 10–12 November 2009. Vaccine. 2010;28:3265–68

4. Kessels JA, Recuenco S, Navarro-Vela AM, et al. Pre-exposure rabies prophylaxis: a systematic review. Bull World Health Organ. 2017;95:210–19C.
5. Brown D, Featherstone JJ, Fooks AR, Gettner S, Lloyd E, Schweiger M. Intradermal pre-exposure rabies vaccine elicits long lasting immunity. Vaccine. 2008;26:3909–12.
6. Narayana A, Manoharan A, Narayan MS, Kalappa SM, Biligumba G, Haradanahalli R, Anand AM. Comparison of safety and immunogenicity of 2 WHO prequalified rabies vaccines administered by one week, 4 site intra dermal regimen (4-4-4-0-0) in animal bite cases. Hum Vaccin Immunother. 2015;11:1748–53.
7. Zhang X, Zhu Z, Wang C. Persistence of rabies antibody 5 years after postexposure prophylaxis with vero cell antirabies vaccine and antibody response to a single booster dose. Clin Vaccine Immunol CVI. 2011;18:1477–147.
8. Malissen B, Tamoutounour S, Henri S. The origins and functions of dendritic cells and macrophages in the skin. Nat Rev Immunol. 2014;14:417–28. https://doi.org/10.1038/nri3683.
9. Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol CVI. 2010;17:1055–65.
10. Rabies vaccines WHO. WHO position paper. Releve Epidemiol Hebd. 2007;82:425–35.
11. Badrane H, Bahloul C, Perrin P, Tordo N. Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. J Virol. 2001;75:3268–76.
12. Brookes SM, Parsons G, Johnson N, McElhinney LM, Fooks AR. Rabies human diploid cell vaccine elicits cross-neutralising and cross-protecting immune responses against European and Australian bat lyssaviruses. Vaccine. 2005;23:4101–9.
13. Malerczyk C, Briggs DJ, Dreesen DW, Banzhoff A. Duration of immunity: an anamnestic response 14 years after rabies vaccination with purified chick embryo cell rabies vaccine. J Travel Med. 2007;14:63–4.
14. Xiang ZQ, Knowles BB, McCarrick JW, Ertl HC. Immune effector mechanisms required for protection to rabies virus. Virology. 1995;214:398–404.
15. Azzoni L, Foulkes AS, Firnhaber C, et al. Antiretroviral therapy interruptions result in loss of protective humoral immunity to neoantigens in HIV-infected individuals. AIDS. 2012;26:1355–62.
16. Celis E, Ou D, Dietzschold B, Koprowski H. Recognition of rabies and rabies-related viruses by T cells derived from human vaccine recipients. J Virol. 1988;62:3128–34.
17. Diebold SS. Recognition of viral single-stranded RNA by Toll-like receptors. Adv Drug Deliv Rev. 2008;60:813–23.
18. Schlee M, Roth A, Hornung V, et al. Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. Immunity. 2009;31:25–34.
19. Lebrun A, Portocarrero C, Kean RB, Barkhouse DA, Faber M. Hooper DC (2015) T-bet is required for the rapid clearance of attenuated rabies virus from central nervous system tissue. J Immunol Baltim Md. 1950;195:4358–68.
20. Kuzmin IV, Hughes GJ, Botvinkin AD, Orciari LA, Rupprecht CE. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. Virus Res. 2005;111:28–43.
21. Tregoning JS, Russell RF, Kinnear E. Adjuvanted influenza vaccines. Hum Vaccin Immunother. 2018;14:550–64. https://doi.org/10.1080/21645515.2017.1415684.
22. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. Lancet. 2004;364:1757–65.
23. Sun X, Belser JA, Pulit-Penaloza JA, et al. Stockpiled pre-pandemic H5N1 influenza virus vaccines with AS03 adjuvant provide cross-protection from H5N2 clade 2.3.4.4 virus challenge in ferrets. Virology. 2017;508:164–9.

24. Wijnans L, Lecomte C, de Vries C, et al. The incidence of narcolepsy in Europe: before, during, and after the influenza A(H1N1)pdm09 pandemic and vaccination campaigns. Vaccine. 2013;31:1246–54.
25. Warrell MJ, Warrell DA, Suntharasamai P, et al. An economical regimen of human diploid cell strain anti-rabies vaccine for post-exposure prophylaxis. Lancet. 1983;2:301–4.
26. Nunberg JH, Doyle MV, York SM, York CJ. Interleukin 2 acts as an adjuvant to increase the potency of inactivated rabies virus vaccine. Proc Natl Acad Sci U S A. 1989;86:4240–3.
27. de Souza Matos DC, Marcovistz R, Neway T, Vieira da Silva AM, Alves EN, Pilet C. Immunostimulatory effects of polar glycopeptidolipids of Mycobacterium chelonae for inactivated rabies vaccine. Vaccine. 2000;18:2125–31.
28. Wang X, Bao M, Wan M, Wei H, Wang L, Yu H, Zhang X, Yu Y, Wang L. A CpG oligodeoxynucleotide acts as a potent adjuvant for inactivated rabies virus vaccine. Vaccine. 2008;26:1893–901.
29. Xiao W, Du L, Liang C, Guan J, Jiang S, Lustigman S, He Y, Zhou Y. Evaluation of recombinant Onchocerca volvulus activation associated protein-1 (ASP-1) as a potent Th1-biased adjuvant with a panel of protein or peptide-based antigens and commercial inactivated vaccines. Vaccine. 2008;26:5022–9.
30. DiStefano D, Antonello JM, Bett AJ, Medi MB, Casimiro DR, ter Meulen J. Immunogenicity of a reduced-dose whole killed rabies vaccine is significantly enhanced by ISCOMATRIX™ adjuvant, Merck amorphous aluminum hydroxylphosphate sulfate (MAA) or a synthetic TLR9 agonist in rhesus macaques. Vaccine. 2013;31:4888–93.
31. Iwaki Y, Sakai Y, Ochiai K, Umemura T, Sunden Y. Enhancement of antibody production against rabies virus by uridine 5′-triphosphate in mice. Microbes Infect. 2014;16:196–202.
32. Su X, Pei Z, Hu S. Ginsenoside Re as an adjuvant to enhance the immune response to the inactivated rabies virus vaccine in mice. Int Immunopharmacol. 2014;20:283–9.
33. Xiao X, Zhang Y, Liu J, Wei Q, Yin X. Immunoenhancement with flagellin as an adjuvant to whole-killed rabies vaccine in mice. Arch Virol. 2016;161:685–91.
34. Zhang W, Zheng X, Cheng N, Gai W, Xue X, Wang Y, Gao Y, Shan J, Yang S, Xia X. Isatis indigotica root polysaccharides as adjuvants for an inactivated rabies virus vaccine. Int J Biol Macromol. 2016;87:7–15.
35. Miao L, Yang Y, Yan M, Li Y, Zhao J, Guo J, Zheng D. Enhanced immune response to rabies viruses by the use of a liposome adjuvant in vaccines. Viral Immunol. 2017; https://doi.org/10.1089/vim.2017.0093.
36. Wijaya L, Tham CYL, Chan YFZ, Wong AWL, Li LT, Wang L-F, Bertoletti A, Low JG. An accelerated rabies vaccine schedule based on toll-like receptor 3 (TLR3) agonist PIKA adjuvant augments rabies virus specific antibody and T cell response in healthy adult volunteers. Vaccine. 2017;35:1175–83.
37. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. Int Rev Immunol. 2011;30:16–34.
38. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124:783–801.
39. Shakin-Eshleman SH, Remaley AT, Eshleman JR, Wunner WH, Spitalnik SL. N-linked glycosylation of rabies virus glycoprotein. Individual sequons differ in their glycosylation efficiencies and influence on cell surface expression. J Biol Chem. 1992;267:10690–8.
40. Fontana D, Kratje R, Etcheverrigaray M, Prieto C. Immunogenic virus-like particles continuously expressed in mammalian cells as a veterinary rabies vaccine candidate. Vaccine. 2015;33:4238–46.
41. Morimoto K, Kawai A, Mifune K. Comparison of rabies virus G proteins produced by cDNA-transfected animal cells that display either inducible or constitutive expression of the gene. J Gen Virol. 1992;73(Pt 2):335–45.
42. Fu ZF, Rupprecht CE, Dietzschold B, Saikumar P, Niu HS, Babka I, Wunner WH, Koprowski H. Oral vaccination of racoons (Procyon lotor) with baculovirus-expressed rabies virusglycoprotein. Vaccine. 1993;11:925–8.

43. Alcock R, Cottingham MG, Rollier CS, et al. Long-term thermostabilization of live poxviral and adenoviral vaccine vectors at supraphysiological temperatures in carbohydrate glass. Sci Transl Med. 2010;2:19ra12.
44. Xiang Z, Li Y, Cun A, Yang W, Ellenberg S, Switzer WM, Kalish ML, Ertl HCJ. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. Emerg Infect Dis. 2006;12:1596–9.
45. Chen H, Xiang ZQ, Li Y, et al. Adenovirus-based vaccines: comparison of vectors from three species of adenoviridae. J Virol. 2010;84:10522–32.
46. Coll JM. The glycoprotein G of rhabdoviruses. Arch Virol. 1995;140:827–51.
47. Rehaud C, Takehara K, Flamand A, Bishop DH. Immunogenic and protective properties of rabies virus glycoprotein expressed by baculovirus vectors. Virology. 1989;173:390–9.
48. Ramya R, Mohana Subramanian B, Sivakumar V, Senthilkumar RL, Sambasiva Rao KRS, Srinivasan VA. Expression and solubilization of insect cell-based rabies virus glycoprotein and assessment of its immunogenicity and protective efficacy in mice. Clin Vaccine Immunol CVI. 2011;18:1673–9.
49. Xiang ZQ, Yang Y, Wilson JM, Ertl HC. A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. Virology. 1996;219:220–7.
50. Astray RM, Ventini DC, Boldorini VLL, Silva FG, Rocca MP, Pereira CA. Rabies virus glycoprotein and immune response pattern using recombinant protein or recombinant RNA viral vectors. Vaccine. 2014;32:2829–32.
51. Klepfer SR, Debouck C, Uffelman J, Jacobs P, Bollen A, Jones EV. Characterization of rabies glycoprotein expressed in yeast. Arch Virol. 1993;128:269–86.
52. Ben Azoun S, Belhaj AE, Göngrich R, Gasser B, Kallel H. Molecular optimization of rabies virus glycoprotein expression in Pichia pastoris. Microb Biotechnol. 2016;9:355–68.
53. Suárez-Patiño SF, Mancini RA, Pereira CA, Suazo CAT, Mendonça RZ, Jorge SAC. Transient expression of rabies virus glycoprotein (RVGP) in Drosophila melanogaster Schneider 2 (S2) cells. J Biotechnol. 2014;192(Pt A):255–62.
54. Qian W, Aguilar F, Wang T, Qiu B. Secretion of truncated recombinant rabies virus glycoprotein with preserved antigenic properties using a co-expression system in Hansenula polymorpha. J Microbiol Seoul Korea. 2013;51:234–40.
55. Xiang Z, Gao G, Reyes-Sandoval A, Cohen CJ, Li Y, Bergelson JM, Wilson JM, Ertl HCJ. Novel, chimpanzee serotype 68-based adenoviral vaccine carrier for induction of antibodies to a transgene product. J Virol. 2002;76:2667–75.
56. Loza-Rubio E, Rojas E, Gómez L, Olivera MTJ, Gómez-Lim MA. Development of an edible rabies vaccine in maize using the Vnukovo strain. Dev Biol. 2008;131:477–82.
57. Ashraf S, Singh PK, Yadav DK, Shahnawaz M, Mishra S, Sawant SV, Tuli R. High level expression of surface glycoprotein of rabies virus in tobacco leaves and its immunoprotective activity in mice. J Biotechnol. 2005;119:1–14.
58. Rojas-Anaya E, Loza-Rubio E, Olivera-Flores MT, Gomez-Lim M. Expression of rabies virus G protein in carrots (Daucus carota). Transgenic Res. 2009;18:911–9.
59. Modelska A, Dietzschold B, Sleysh N, Fu ZF, Steplewski K, Hooper DC, Koprowski H, Yusibov V. Immunization against rabies with plant-derived antigen. Proc Natl Acad Sci U S A. 1998;95:2481–5.
60. Yusibov V, Hooper DC, Spitsin SV, et al. Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine. Vaccine. 2002;20:3155–64.
61. Liu R, Wang J, Yang Y, Khan I, Dong Y, Zhu N. A novel rabies virus lipopeptide provides a better protection by improving the magnitude of DCs activation and T cell responses. Virus Res. 2016;221:66–73.
62. Niu Y, Liu Y, Yang L, Qu H, Zhao J, Hu R, Li J, Liu W. Immunogenicity of multi-epitope-based vaccine candidates administered with the adjuvant Gp96 against rabies. Virol Sin. 2016;31:168–75.
63. Schnell MJ, Tan GS, Dietzschold B. The application of reverse genetics technology in the study of rabies virus (RV) pathogenesis and for the development of novel RV vaccines. J Neurovirol. 2005;11:76–81.

64. Morimoto K, Shoji Y, Inoue S. Characterization of P gene-deficient rabies virus: propagation, pathogenicity and antigenicity. Virus Res. 2005;111:61–7.
65. Cenna J, Tan GS, Papaneri AB, Dietzschold B, Schnell MJ, McGettigan JP. Immune modulating effect by a phosphoprotein-deleted rabies virus vaccine vector expressing two copies of the rabies virus glycoprotein gene. Vaccine. 2008;26:6405–14.
66. Ito N, Sugiyama M, Yamada K, Shimizu K, Takayama-Ito M, Hosokawa J, Minamoto N. Characterization of M gene-deficient rabies virus with advantages of effective immunization and safety as a vaccine strain. Microbiol Immunol. 2005;49:971–9.
67. Hooper DC, Phares TW, Fabis MJ, Roy A. The production of antibody by invading B cells is required for the clearance of rabies virus from the central nervous system. PLoS Negl Trop Dis. 2009;3:e535.
68. Liu X, Yang Y, Sun Z, Chen J, Ai J, Dun C, Fu ZF, Niu X, Guo X. A recombinant rabies virus encoding two copies of the glycoprotein gene confers protection in dogs against a virulent challenge. PloS One. 2014;9:e87105.
69. Xiang ZQ, Greenberg L, Ertl HC, Rupprecht CE. Protection of non-human primates against rabies with an adenovirus recombinant vaccine. Virology. 2014;450–451:243–9.
70. Ge J, Wang X, Tao L, Wen Z, Feng N, Yang S, Xia X, Yang C, Chen H, Bu Z. Newcastle disease virus-vectored rabies vaccine is safe, highly immunogenic, and provides long-lasting protection in dogs and cats. J Virol. 2011;85:8241–52.
71. Wu Q, Yu F, Xu J, Li Y, Chen H, Xiao S, Fu ZF, Fang L. Rabies-virus-glycoprotein-pseudotyped recombinant baculovirus vaccine confers complete protection against lethal rabies virus challenge in a mouse model. Vet Microbiol. 2014;171:93–101.
72. Chen Z, Zhou M, Gao X, Zhang G, Ren G, Gnanadurai CW, Fu ZF, He B. A novel rabies vaccine based on a recombinant parainfluenza virus 5 expressing rabies virus glycoprotein. J Virol. 2013;87:2986–93.
73. Reyes-Sandoval A, Ertl HC. DNA vaccines. Curr Mol Med. 2001;1:217–43.
74. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success. Curr Opin Immunol. 2011;23:421–9.
75. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol. 2002;20:709–60.
76. Kibuuka H, Berkowitz NM, Millard M, 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. 2015;385:1545–54.
77. Liu MA, Ulmer JB. Human clinical trials of plasmid DNA vaccines. Adv Genet. 2005;55:25–40.
78. Ledgerwood JE, Bellamy AR, Belshe R, et al. DNA priming for seasonal influenza vaccine: a phase 1b double-blind randomized clinical trial. PloS One. 2015;10:e0125914.
79. Xiang ZQ, Spitalnik S, Tran M, Wunner WH, Cheng J, Ertl HC. Vaccination with a plasmid vector carrying the rabies virus glycoprotein gene induces protective immunity against rabies virus. Virology. 1994;199:132–40.
80. Ray NB, Ewalt LC, Lodmell DL. Nanogram quantities of plasmid DNA encoding the rabies virus glycoprotein protect mice against lethal rabies virus infection. Vaccine. 1997;15:892–5.
81. Lodmell DL, Ray NB, Ewalt LC. Gene gun particle-mediated vaccination with plasmid DNA confers protective immunity against rabies virus infection. Vaccine. 1998;16:115–8.
82. Bahloul C, Jacob Y, Tordo N, Perrin P. DNA-based immunization for exploring the enlargement of immunological cross-reactivity against the lyssaviruses. Vaccine. 1998;16:417–25.
83. Lodmell DL, Parnell MJ, Bailey JR, Ewalt LC, Hanlon CA. One-time gene gun or intramuscular rabies DNA vaccination of non-human primates: comparison of neutralizing antibody responses and protection against rabies virus 1 year after vaccination. Vaccine. 2001;20:838–44.

84. Osorio JE, Tomlinson CC, Frank RS, Haanes EJ, Rushlow K, Haynes JR, Stinchcomb DT. Immunization of dogs and cats with a DNA vaccine against rabies virus. Vaccine. 1999;17:1109–16.
85. Xiang Z, Ertl HC. Manipulation of the immune response to a plasmid-encoded viral antigen by coinoculation with plasmids expressing cytokines. Immunity. 1995;2:129–35.
86. Garg R, Kaur M, Saxena A, Prasad R, Bhatnagar R. Alum adjuvanted rabies DNA vaccine confers 80% protection against lethal 50 LD50 rabies challenge virus standard strain. Mol Immunol. 2017;85:166–73.
87. Lodmell DL, Ray NB, Ulrich JT, Ewalt LC. DNA vaccination of mice against rabies virus: effects of the route of vaccination and the adjuvant monophosphoryl lipid A (MPL). Vaccine. 2000;18:1059–66.
88. Fischer L, Minke J, Dufay N, Baudu P, Audonnet JC. Rabies DNA vaccine in the horse: strategies to improve serological responses. Vaccine. 2003;21:4593–6.
89. Margalith M, Vilalta A. Sustained protective rabies neutralizing antibody titers after administration of cationic lipid-formulated pDNA vaccine. Genet Vaccines Ther. 2006;4:2.
90. Kaur M, Saxena A, Rai A, Bhatnagar R. Rabies DNA vaccine encoding lysosome-targeted glycoprotein supplemented with Emulsigen-D confers complete protection in preexposure and postexposure studies in BALB/c mice. FASEB J Off Publ Fed Am Soc Exp Biol. 2010;24:173–83.
91. Spearman P, Mulligan M, Anderson EJ, Shane AL, Stephens K, Gibson T, Hartwell B, Hannaman D, Watson NL, Singh K. A phase 1, randomized, controlled dose-escalation study of EP-1300 polyepitope DNA vaccine against Plasmodium falciparum malaria administered via electroporation. Vaccine. 2016;34:5571–8. https://doi.org/10.1016/j.vaccine.2016.09.041.
92. Elizaga ML, Li SS, Kochar NK, Wilson GJ, Allen MA, Tieu HVN, Frank I, Sobieszczyk ME, Cohen KW, Sanchez B, Latham TE, Clarke DK, Egan MA, Eldridge JH, Hannaman D, Xu R, Ota-Setlik A, McElrath MJ, Hay CM, NIAID HIV Vaccine Trials Network (HVTN) 087 Study Team. Safety and tolerability of HIV-1 multiantigen pDNA vaccine given with IL-12 plasmid DNA via electroporation, boosted with a recombinant vesicular stomatitis virus HIV Gag vaccine in healthy volunteers in a randomized, controlled clinical trial. PLoS One. 2018;13(9): e0202753. https://doi.org/10.1371/journal.pone.0202753.. eCollection 2018
93. Tuyishime S, Haut LH, Kurupati RK, Billingsley JM, Carnathan D, Gangahara S, Styles TM, Xiang Z, Li Y, Zopfs M, Liu Q, Zhou X, Lewis MG, Amara RR, Bosinger S, Silvestri G, Ertl HCJ. Correlates of protection against SIV_{mac251} infection in rhesus macaques immunized with chimpanzee-derived adenovirus vectors. EBioMedicine. 2018;31:25–35. https://doi.org/10.1016/j.ebiom.2018.02.025.
94. Maki J, Guiot AL, Aubert M, Brochier B, Cliquet F, Hanlon CA, King R, Oertli EH, Rupprecht CE, Schumacher C, Slate D, Yakobson B, Wohlers A, Lankau EW. Oral vaccination of wildlife using a vaccinia-rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. Vet Res. 2017;48:57. https://doi.org/10.1186/s13567-017-0459-9.
95. Hooper JW, Moon JE, Paolino KM, Newcomer R, McLain DE, Josleyn M, Hannaman D, Schmaljohn C. A Phase 1 clinical trial of Hantaan virus and Puumala virus M-segment DNA vaccines for haemorrhagic fever with renal syndrome delivered by intramuscular electroporation. Clin Microbiol Infect. 2014;20(Suppl 5):110–7. https://doi.org/10.1111/1469-0691.12553.
96. Kibuuka H, Berkowitz NM, Millard M, Enama ME, Tindikahwa A, Sekiziyivu AB, Costner P, Sitar S, Glover D, Hu Z, Joshi G, Stanley D, Kunchai M, Eller LA, Bailer RT, Koup RA, Nabel GJ, Mascola JR, Sullivan NJ, Graham BS, Roederer M, Michael NL, Robb ML, Ledgerwood JE, RV 247 Study Team. 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. 2015;385:1545–54. https://doi.org/10.1016/S0140-6736(14)62385-0.

97. Taylor J, Tartaglia J, Rivière M, Duret C, Languet B, Chappuis G, Paoletti E. Applications of canarypox (ALVAC) vectors in human and veterinary vaccination. Dev Biol Stand. 1994;82:131–5.
98. Kardani K, Bolhassani A, Shahbazi S. Prime-boost vaccine strategy against viral infections: mechanisms and benefits. Vaccine. 2016;34:413–23. https://doi.org/10.1016/j.vaccine.2015.11.062.
99. De Santis O, Audran R, Pothin E, 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. Lancet Infect Dis. 2016;16:311–20.
100. Taylor J, Trimarchi C, Weinberg R, Languet B, Guillemin F, Desmettre P, Paoletti E. Efficacy studies on a canarypox-rabies recombinant virus. Vaccine. 1991;9:190–3.
101. Fries LF, Tartaglia J, Taylor J, Kauffman EK, Meignier B, Paoletti E, Plotkin S. Human safety and immunogenicity of a canarypox-rabies glycoprotein recombinant vaccine: an alternative poxvirus vector system. Vaccine. 1996;14:428–34.
102. Amann R, Rohde J, Wulle U, Conlee D, Raue R, Martinon O, Rziha H-J. A new rabies vaccine based on a recombinant ORF virus (parapoxvirus) expressing the rabies virus glycoprotein. J Virol. 2013;87:1618–30.
103. Schnee M, Vogel AB, Voss D, Petsch B, Baumhof P, Kramps T, Stitz L. An mRNA vaccine encoding rabies virus glycoprotein induces protection against lethal infection in mice and correlates of protection in adult and newborn pigs. PLoS Negl Trop Dis. 2016;10:e0004746.
104. Alberer M, Gnad-Vogt U, Hong HS, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet. 2017;390:1511–20.
105. Xiang ZQ, Pasquini S, Ertl HC. Induction of genital immunity by DNA priming and intranasal booster immunization with a replication-defective adenoviral recombinant. J Immunol. 1999;162:6716–23.
106. Bahloul C, Ahmed SBH, B’chir BI, Kharmachi H, Hayouni EA, Dellagi K. Post-exposure therapy in mice against experimental rabies: a single injection of DNA vaccine is as effective as five injections of cell culture-derived vaccine. Vaccine. 2003;22:177–84.
107. Lodmell DL, Ewalt LC. Post-exposure DNA vaccination protects mice against rabies virus. Vaccine. 2001;19:2468–73.
108. Lodmell DL, Parnell MJ, Bailey JR, Ewalt LC, Hanlon CA. Rabies DNA vaccination of non-human primates: post-exposure studies using gene gun methodology that accelerates induction of neutralizing antibody and enhances neutralizing antibody titers. Vaccine. 2002;20:2221–8.
109. Tesoro Cruz E, Feria Romero IA, López Mendoza JG, Orozco Suárez S, Hernández González R, Favela FB, Pérez Torres A, Aguilar-Setién Á. Efficient post-exposure prophylaxis against rabies by applying a four-dose DNA vaccine intranasally. Vaccine. 2008;26:6936–44.
110. Saxena S, Dahiya SS, Sonwane AA, Patel CL, Saini M, Rai A, Gupta PK. A sindbis virus replicon-based DNA vaccine encoding the rabies virus glycoprotein elicits immune responses and complete protection in mice from lethal challenge. Vaccine. 2008;26:6592–601.
111. Rampling T, Ewer KJ, Bowyer G, et al. Safety and high level efficacy of the combination malaria vaccine regimen of RTS, S/AS01B with chimpanzee Adenovirus 63 and modified vaccinia Ankara vectored vaccines expressing ME-TRAP. J Infect Dis. 2016;214:772–81.
112. Afolabi MO, Tiono AB, Adetifa UJ, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in West African children and infants. Mol Ther. 2016;24:1470–7.
113. Rosenthal SR, Merchlinsky M, Kleppinger C, Goldenthal KL. Developing new smallpox vaccines. Emerg Infect Dis. 2001;7:920–6.
114. McCoy K, Tatsis N, Korioth-Schmitz B, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. J Virol. 2007;81:6594–604.

115. Roberts DM, Nanda A, Havenga MJE, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. Nature. 2006;441:239–43.
116. Giel-Moloney M, Rumyantsev AA, David F, Figueiredo M, Feilmeier B, Mebatsion T, Parrington M, Kleanthous H, Pugachev KV. A novel approach to a rabies vaccine based on a recombinant single-cycle flavivirus vector. Vaccine. 2017;35(49 Pt B):6898–904. https://doi.org/10.1016/j.vaccine.2017.08.055.
117. Rupprecht CE, Wiktor TJ, Johnston DH, Hamir AN, Dietzschold B, Wunner WH, Glickman LT, Koprowski H. Oral immunization and protection of raccoons (Procyon lotor) with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proc Natl Acad Sci U S A. 1986;83:7947–50.
118. Thomas I, Brochier B, Languet B, Blancou J, Peharpre D, Kieny MP, Desmettre P, Chappuis G, Pastoret PP. Primary multiplication site of the vaccinia-rabies glycoprotein recombinant virus administered to foxes by the oral route. J Gen Virol. 1990;71(Pt 1):37–42.
119. Weyer J, Rupprecht CE, Mans J, Viljoen GJ, Nel LH. Generation and evaluation of a recombinant modified vaccinia virus Ankara vaccine for rabies. Vaccine. 2007;25:4213–22.
120. Rupprecht CE, Blass L, Smith K, Orciari LA, Niezgoda M, Whitfield SG, Gibbons RV, Guerra M, Hanlon CA. Human infection due to recombinant vaccinia-rabies glycoprotein virus. N Engl J Med. 2001;345(8):582–6.
121. Baden LR, Walsh SR, Seaman MS, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). J Infect Dis. 2013;207:240–7.
122. Barnes E, Folgori A, Capone S, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med. 2012;4:115ra1.
123. van Zyl-Smit RN, Esmail A, Bateman ME, et al. Safety and immunogenicity of Adenovirus 35 tuberculosis vaccine candidate in adults with active or previous tuberculosis. A Randomized Trial. Am J Respir Crit Care Med. 2017;195:1171–80.
124. Yarosh OK, Wandeler AI, Graham FL, Campbell JB, Prevec L. Human adenovirus type 5 vectors expressing rabies glycoprotein. Vaccine. 1996;14:1257–64.
125. Ghebremedhin B. Human adenovirus: Viral pathogen with increasing importance. Eur J Microbiol Immunol (Bp). 2014;4:26–33. https://doi.org/10.1556/EuJMI.4.2014.1.2.
126. Tatsis N, Fitzgerald JC, Reyes-Sandoval A, et al. Adenoviral vectors persist in vivo and maintain activated $CD8^+$ T cells: implications for their use as vaccines. Blood. 2007;110:1916–23.
127. 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;25:318–27.
128. Afkhami S, LeClair DA, Haddadi S, Lai R, Toniolo SP, Ertl HC, Cranston ED, Thompson MR, Xing Z. Spray dried human and chimpanzee adenoviral-vectored vaccines are thermally stable and immunogenic in vivo. Vaccine. 2017;35:2916–24.