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Viral vector-based vaccines against SARS-CoV-2

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Abstract

Viral vectors have been frequently applied for vaccine development. It has also been the case for vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to tackle the coronavirus disease 2019 (COVID-19) pandemic. A multitude of different viral vectors have been mainly targeting the SARS-CoV-2 spike (S) protein as antigen. Intramuscular injection has been most commonly used, but also intranasal administration has been tested. Adenovirus vector-based vaccines are the most advanced with several vaccines receiving Emergency Use Authorization (EUA). The simian ChAdOx1 nCoV-19 vaccine applied as a prime-boost regimen has provided 62.1-90% vaccine efficacy in clinical trials. The Ad26.COV2.S vaccine requires only one immunization to provide protection against SARS-CoV-2. The rAd26-S/rAd5-S vaccine utilizes the Ad26 serotype for the prime immunization followed by a boost with the Ad5 serotype resulting in 91.2% vaccine efficacy. All adenovirus-based vaccines are used for mass vaccinations. Moreover, vaccine candidates based on vaccinia virus and lentivirus vectors have been subjected to clinical evaluation. Among self-replicating RNA viruses, vaccine vectors based on measles virus, rhabdoviruses, and alphaviruses have been engineered and tested in clinical trials. In addition to the intramuscular route of administration vaccine candidates based on influenza viruses and adenoviruses have been subjected to intranasal delivery showing antibody responses and protection against SARS-CoV-2 challenges in animal models. The detection of novel more transmissible and pathogenic SARS-CoV-2 variants added concerns about the vaccine efficacy and needs to be monitored. Moreover, the cause of recently documented rare cases of vaccine-induced immune thrombotic thrombocytopenia (VITT) must be investigated.

Keywords

Viral vectors, vaccines, SARS-CoV-2, COVID-19, clinical trials, protection

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic has triggered an intensive race to develop numerous vaccine candidates targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. No stones were left unturned as all potential approaches such as vaccines based on inactivated and attenuated viruses, protein subunits, nucleic acids and viral vectors have been evaluated [2]. The first COVID-19 vaccine

to receive Emergency Use Authorization (EUA) was the BNT162b2 mRNA-based vaccine, which has now been subjected to mass vaccinations around the world [3]. It was quickly followed by another mRNA-based vaccine, mRNA-1273 [4]. Vaccines based on inactivated viruses have also recently been approved in China [5]. Moreover, for protein subunit vaccine candidates [6] and DNA-based vaccine candidates [7], promising results have been obtained in clinical trials. Viral vectors have previously been used frequently for vaccine development [8] and COVID-19 vaccines are no exception. This review focuses uniquely on viral vector-based COVID-19 vaccines, describing their specific features, preclinical studies in animal models, and clinical evaluation in humans. The effect on the efficacy of viral vector-based vaccines by the recently discovered SARS-CoV-2 variants and vaccine-induced immune thrombotic thrombocytopenia (VITT) are also discussed.

Viral vector-based COVID-19 vaccines

Various viral delivery vectors based on adenoviruses, vaccinia viruses, lentiviruses, measles virus, rhabdoviruses, alphaviruses and influenza viruses have been engineered for SARS-CoV-2 vaccines and subjected to preclinical studies in animal models and clinical evaluation in healthy volunteers (Table 1). The common feature of the vaccine candidates based on viral vectors is the utilization of the SARS-CoV-2 spike (S) protein as the antigen although in some applications the SARS-CoV-2 nucleocapsid (N) protein has also been targeted. The route of administration has in the majority of applications focused on intramuscular administration. However, as SARS-CoV-2 infections occur through the respiratory tract [9], intranasal delivery has been considered as an attractive alternative. In this context, both adenovirus- and influenza virus-based vectors have been developed.

Table 1. Preclinical and clinical studies for viral vector-based COVID-19 vaccines

Vector	Construct	Findings	Ref
ChAdOx1	ChAdOx1 nCoV-19	Protection against SARS-CoV-2 in macaques	[11]
ChAdOx1	ChAdOx1 nCoV-19	Protection after intranasal injection in mice	[11]
ChAdOx1	ChAdOx1 nCoV-19	Phase I/II: safety, strong immune responses	[12]
ChAdOx1	ChAdOx1 nCoV-19	Phase II/III: high Ab titers in all age groups	[13]
ChAdOx1	ChAdOx1 nCoV-19	Phase III: > 30,000 healthy volunteers	[14]
ChAdOx1	ChAdOx1 nCoV-19	Phase III: 62.1-90.0% vaccine efficacy	[15]
ChAdOx1	ChAdOx1 nCoV-19	EUA in the UK in December 2020	[16]
Ad26	Ad26.COV2.S	Protection of hamsters after single injection	[18]
Ad26	Ad26.COV2.S	Protection of macaques after single injection	[19]
Ad26	Ad26.COV2.S	Phase I: single injection > immune response	[20]
Ad26	Ad26.COV2.S	Phase I/II: strong immune response	[21]
Ad26	Ad26.COV2.S	Phase III: > 60,000 healthy volunteers	[22]
Ad26	Ad26.COV2.S	EUA by the FDA in February 2021	[23]
Ad26/Ad5	rAd26-S/rAd5-S	100% protection in mice, hamsters & primates	[24]
Ad26/Ad5	rAd26-S/rAd5-S	Phase I/II: safety, strong immune response	[25]
Ad26/Ad5	rAd26-S/rAd5-S	Phase III: 91.6% vaccine efficacy	[26]
Ad26/Ad5	rAd26-S/rAd5-S	EUA in Russia in July 2020	[27]
Ad5	Ad5-S-nb2	SARS-CoV-2 protection in mice & ferrets	[28]
Ad5	Ad5-S.nb2	SARS-CoV-2 protection in macaques	[29]
Ad5	Ad5-S.nb2	Phase I: binding and neutralization Abs	[30]
Ad5	Ad5-S.nb2	Phase II: Abs dependent on age, pre-existing Ad5	[31]
Ad5	Ad5-S.nb2	90.1% efficacy of preventing severe COVID-19	[32]
Ad5	Ad5-S.nb2	EUA in China in February 2021	[33]
Ad5	Ad5-S nb2	Protection in macaques after i.n. administration	[29]
Ad5	AdCOVID-RBD	Systemic & mucosal immunity after i.n. delivery	[59]
Ad5	VXA-COV2-1	Strong immune response after i.n. delivery in mice	[66]
Ad5	VXA-COV2-1	Phase I: oral delivery, strong immunogenicity	[69]
GRAd	GRAd-COV2	Robust immune responses in mice & macaques	[34]
Grad	GRAd-COV2	Phase I: study in progress	[35]

Table 1. Preclinical and clinical studies for viral vector-based COVID-19 vaccines (*continued*)

Vector	Construct	Findings	Ref
ChAd68	ChAdV68 + SAM	Phase I: study in progress	[37]
AAVCOVID-1	AAV2-CoV-2 S	Protection in primates after single administration	[38]
MVA	MVA-CoV-2 S/N	Humoral & cellular immune responses in mice	[40]
MVA	MVA-COV-2 S	Protection in mice	[41]
MVA	MVA-COV-2	Phase I: study in progress	[42]
MVA	MVA-COV-2	Phase I: study in progress	[43]
NDV	NDV-SARS-CoV-2 S	Protection in mice and hamsters	[45]
NDV	NDV-SARS-CoV-2 S	Protection in mice	[46]
NDV	NDV-HXP-S	Phase I/II: study in progress	[47]
MV	MV-SARS-CoV-2-S	Th1-biased and T-cell immune responses in mice	[49]
MV	MV-SARS-CoV-2-S	Phase I: weak immunogenicity, trial discontinued	[51]
VSV	VSV-SARS-CoV-2-S	Protection against SARS-CoV-2 pathogenesis in mice	[52]
VSV	VSV-SARS-CoV-2-S	Phase I: weak immunogenicity, trial discontinued	[53]
VSV	VSV-ΔG	Protection against SARS-CoV-2 in hamsters	[55]
VSV	VSV-ΔG	Phase I/II: study in progress	[56]
VEE RNA	VEE-SARS-CoV-2 S	Th1-biased immune response in mice	[58]
VEE RNA	VEE-SARS-CoV-2 S	Phase I: dose-escalation study in progress	[59]
LV	LV-SARS-COV-2-S	Reduced viral load after i.n. delivery in mice	[61]
LV	LV-SMENP + CTL	Phase I/II: s.c. delivery of LV-DCs. i.v. CTLs	[62]
Influenza	ΔNA(RBD)-Flu	Single i.n. dose: strong immune response	[63]
Influenza	IFV-COV-2 S RBD	Phase I: registered trial for intranasal spray	[64]
Influenza	IFV-COV-2 S RBD	Phase II: registered trial for intranasal spray	[65]

AAV: adeno-associated virus; Ab: antibodies; CTLs: cytotoxic T cell lymphocytes; DCs: dendritic cells; FDA: Food and Drug Administration; GRAd: gorilla adenovirus; i.n.: intranasal; IFV: influenza virus; i.v.: intravenous; LV: lentivirus; LV-SMENP: lentivirus vector expressing SARS-CoV-2 minigene; MV: measles virus; MVA: modified vaccinia virus Ankara; NDV: Newcastle disease virus; RBD: receptor binding domain; SAM: self-amplifying mRNA: s.c.: subcutaneous; VEE: Venezuelan equine encephalitis virus; VSV: vesicular stomatitis virus; VSV-ΔG: VSV G protein

Adenovirus and adeno-associated virus vector-based vaccines

Adenovirus vectors have a long tradition as gene transfer and vaccine vectors and particularly the second and third generation adenovirus vectors have demonstrated high safety levels and good delivery efficacy [10]. The codon-optimized SARS-CoV-2 S protein has been utilized as the common antigen although, different strategies related to vector engineering have been applied. In one approach, the simian adenovirus vector ChAdOx1 was utilized to avoid any pre-existing adenovirus immunity in humans. The ChAdOx1 nCoV-19 vaccine candidate showed protection in immunized rhesus macaques [11]. The positive findings from preclinical studies in rodents and non-human primates supported the transfer to clinical trials with the ChAdOx1 nCoV-19 vaccine candidate. Good safety and both humoral and cellular immune responses were obtained in a phase I/II clinical trial [12]. Furthermore, interim results from a phase II/III study showed more frequent adverse events in younger individuals, but the elicited neutralizing antibody titers were similar for all age groups (18-15 years, 56-69 years, and 70 years and older) [13]. Additionally, phase III clinical evaluation in more than 30,000 volunteers have been conducted [14]. Interim phase III results from the UK, Brazil, and South Africa showed good vaccination safety and 62.1% vaccine efficacy after two vaccinations with 5×10^{10} ChAdOx1 nCoV-19 particles and up to 90% in individuals receiving a prime dose of 2.2×10^{10} particles and a boost of 5.5×10^{10} particles [15]. The ChAdOx1 nCoV-19 vaccine received an EUA in the UK in December 2020 [16]. In contrast to the ChAdOx1 nCoV-19 vaccine, the Ad26.COV2.S vaccine is based on the human Ad26 serotype expressing the prefusion-stabilized SARS-CoV-2 S protein, and requires only one immunization [17]. This was confirmed in hamsters, where a single injection of Ad26.COV2.S elicited neutralizing antibodies and protected the animals from SARS-CoV-2-associated pneumonia and death [18]. Moreover, a single immunization of macaques elicited strong neutralizing antibody responses and provided protection against SARS-CoV-2 challenges [19]. In the context of clinical trials, a single administration of Ad26.COV2.S elicited rapid binding, neutralization antibody responses and cellular immune responses in a phase I study in 25 healthy volunteers [20]. Moreover,

1,045 healthy volunteers were vaccinated with a single dose of 1×10^{10} or 5×10^{10} Ad26.COV2.S particles in a phase I/II study showing good safety and strong immune responses [21]. The Ad26.COV2.S vaccine has been subjected to large phase III clinical trials with 60,000 participants [22] and received an EUA by the FDA in February 2021 [23].

As mentioned earlier, simian adenovirus vectors have been used for SARS-CoV-2 vaccine development to address any potential pre-existing immunity against human adenoviruses in the population [11]. However, as the current adenovirus-based vaccines except for Ad26.COV2.S [18] require a prime-boost regimen, neutralizing antibodies against adenoviruses might reduce the efficacy of a second or a third immunization with the same adenovirus serotype. For this reason, a strategy of prime vaccination with an Ad26 serotype vector expressing the SARS-CoV-2 S protein followed by a booster vaccination with another adenovirus serotype, the Ad5 expressing the SARS-CoV-2 S protein, was evaluated.

In preclinical studies the rAd26-S/rAd5-S vaccine candidate showed 100% protection in transgenic mice, hamsters, and primates [24]. Moreover, good safety, mild adverse events, and robust immune responses were observed in a phase I/II clinical trial [25]. Interim results from a phase III study with the rAd26-S/rAd5-S vaccine showed good tolerability and 91.6% vaccine efficacy [26]. The rAd26-S/rAd5-S (Sputnik V) vaccine received an EUA in Russia already in July 2020 although only preliminary vaccine evaluation had been conducted in 76 volunteers [27].

A third generation Ad5 serotype vector expressing the SARS-CoV-2 S protein (Ad5-S-nb2) was intramuscularly administered into mice and ferrets, which resulted in protection against challenges with SARS-CoV-2 [28]. Moreover, the Ad5-S-nb2 vaccine provided projection against SARS-CoV-2 in rhesus macaques [29]. In the case of clinical trials, a single dose of Ad5-S-nb2 induced both binding and neutralizing antibodies in healthy volunteers [30]. However, the level of response depended on pre-existing Ad5 antibodies and the age of the vaccinated person [31]. Interim results from a phase III trial indicated that a single dose of the Ad5-S-nb2 vaccine showed an overall efficacy of 65.3% of preventing all symptomatic COVID-19 disease 28 days post-vaccination [32]. Moreover, Ad5-S-nb2 showed a 90.1% efficacy of preventing severe COVID-19 disease 28 days post-immunization. The Ad5-S-nb2 received an EUA in February 2021 in China [33] and further in Mexico, Pakistan, and Hungary.

The gorilla adenovirus GRAd has been used for the expression of the prefusion stabilized SARS-CoV-2 S protein [34]. The GRAd-COV2 vaccine candidate elicited robust immunogenicity in both mice and macaques. The functional antibodies neutralized SARS-CoV-2 infection, blocked SARS-CoV-2 S protein binding to angiotensin-converting enzyme 2 (ACE2) and generated robust T helper 1 (Th1)-dominated cellular responses. The GRAd-COV2 vaccine candidate is currently in phase I evaluation [35]. In another vaccine approach, the chimpanzee adenovirus serotype 68 (ChAdV68) [36] was combined in a prime-boost regimen with a SAM expressing the SARS-CoV-2 S protein and T-cell epitopes from the SARS-CoV-2 N protein. A dose-escalation phase I clinical trial with a ChAdV68 prime vaccination and SAM boost vaccination is in progress [37].

Recently, the AAV vector-based vaccine candidate AAVCOVID-1 was introduced [38]. The SARS-CoV-2 S gene was expressed from an AAV2 inverted terminal repeat (ITR) with an AAVrh32.33 capsid, showing potent immunogenicity in mice and non-human primates. Moreover, a single immunization provided complete protection in macaques challenged with SARS-CoV-2. Neutralizing antibodies were sustained for a year. Neither pre-existing immunity against AAVCOVID-1 in humans nor cross-reactivity to common AAV vectors used in gene therapy were detected. Single dose administration, high yield manufacturing and stability for one month at room temperature make the AAV-based approach attractive for potential global use once efficacy has been confirmed in clinical trials.

Vaccinia virus vector-based vaccines

Poxviruses, especially vaccinia viruses and the MVA strain, have been regularly applied for vaccine development [39]. In the context of COVID-19 vaccines, a synthetic MVA-based vaccine platform has been engineered for co-expression of SARS-CoV-2 S and N proteins [40]. Mice immunized with the synthetic MVA (sMVA) vector induced robust SARS-CoV-2 antigen-specific humoral and cellular immune

responses. In another study, mice subjected to a prime-boost regimen with either DNA/MVA-COV2-S or MVA-COV2-S/MVA-COV2-S showed broad SARS-CoV-2 S-specific $CD4^+$ and $CD8^+$ T-cell responses and also induced high immunoglobulin G (IgG) titers [41]. Immunization prevented viral replication in mouse lung and protected humanized K18-hACE2 mice from SARS-CoV-2 challenges. MVA-based vaccines have been subjected to the first-in-human phase I study [42]. Safety and tolerability are evaluated in healthy volunteers receiving two doses of either 1 x 10^7 or 1 × 10^8 IU of MVA-SARS-COV-2 S. In another phase I study, a synthetic MVA vector expressing different regions of the SARS-CoV-2 genome is administered at doses of 1 × 10^7, 1 × 10^8, and 2.5 × 10^8 pfu to 129 healthy volunteers for safety and tolerability analysis [43].

Orthoavulavirus-based vaccines

NDV vectors have previously been confirmed as attractive delivery vehicles in oncology [44]. In the context of COVID-19, application of a chimeric NDV vector generated stable expression of the membrane anchored SARS-CoV-2 S protein [45]. Immunization of mice and hamsters elicited robust binding and neutralizing antibodies and provided protection against SARS-CoV-2. Moreover, expression of wildtype and membrane-anchored forms of the SARS-CoV-2 S protein from an NDV vector induced strong antibody responses and protected mice from SARS-CoV-2 infections [46]. A phase I/II clinical trial in healthy volunteers receiving the NDV-SARS-CoV-2 S vector (NDV-HXP-S) has started [47].

Self-amplifying RNA virus vector-based vaccines

Self-amplifying RNA viruses such as alphaviruses, flaviviruses, measles viruses, and rhabdoviruses, are the building blocks for the SAM technology [48]. The special feature of SAM comprises of the non-structural protein (nsPs) genes encoding for the replicase complex in alphaviruses and flaviviruses responsible for an estimated 200,000-fold amplification of viral RNA in infected host cells. In contrast, the RNA-dependent RNA polymerase (RdRp) is located in the structural genes of MV and rhabdoviruses, providing self-replication of viral RNA. For this reason, the SAM technology became an attractive alternative for vaccine development. Among self-replicating RNA viruses, MV vectors have been engineered for the expression of the full-length SARS-CoV-2 S protein [49]. A prime-boost immunization of mice with MV-SARS-CoV-2-S elicited high levels of effective Th1-biased antibody and T-cell responses. Protective S-specific IgG antibodies and multifunctional $CD4^+$ and $CD8^+$ T-cell responses were also detected. The MV-SARS-CoV-2-S vaccine candidate TMV-083 was evaluated in 90 volunteers in a phase I clinical trial [50]. Unfortunately, initial findings were disappointing as the immune responses in vaccinated volunteers were weaker than detected in convalescent COVID-19 patients, leading to the termination of the trial [51]. In another approach, a replication-competent VSV vector was engineered to express the SARS-CoV-2 S protein [52]. Immunization of BALB/c mice generated neutralizing antibody responses and provided protection from SARS-CoV-2-related pathogenesis. The VSV-SARS-CoV-2 S vaccine candidate V590 has been subjected to a phase I trial to evaluate its safety and tolerability in 252 volunteers [53]. Despite good tolerability the trial was discontinued as the immune responses in vaccinated individuals were weaker than those seen in convalescent COVID-19 patients [54].

In another approach, the G protein on the surface of replication-competent VSV particles was replaced by the SARS-CoV-2 S protein resulting in the chimeric VSV-ΔG vector [55]. A single dose of 5 × 10^6 pfu of VSV-ΔG elicited potent neutralizing antibodies and protected golden Syrian hamsters from challenges with SARS-CoV-2. Neither lung damage nor presence of viral load were detected in immunized hamsters. The VSV-ΔG vaccine has entered a phase I/II clinical evaluation in 18-55-year-old volunteers receiving a single dose of 5 × 10^5, 5 × 10^6 and 5 × 10^7 pfu of VSV-ΔG in part one of the study [56]. The focus in the second part will be on elderly volunteers, who will receive a single injection as in part one or two injections of 5 × 10^5 pfu at 28 days interval. While the study was on-going, the EUA for the BNT162b2 mRNA vaccine was received, which resulted in an ethical and executional dilemma concerning the placebo arm of the phase I/II trial with the VSV-ΔG vaccine [57]. It was concluded that the placebo arm was critical for study quality, and it was decided that a follow-up prior to unblinding of 56 days provided a reasonable balance between ethics and execution. The individuals who received placebo were offered either an approved vaccine outside the study or to re-consent to the study with a 1:3 chance to receive placebo.

An alternative approach based on SAM technology relates to the application of alphavirus RNA replicons. For instance, the Venezuelan equine encephalitis virus (VEE) RNA replicon expressing the prefusion-stabilized SARS-CoV-2 S protein was encapsulated in lipid nanoparticles (LNPs) [58]. Immunization of BALB/c mice with LNP-SARS-CoV-2 S RNA elicited dose-dependent SARS-CoV-2-specific antibody responses and neutralization of virus. The Th1-biased antibody responses were superior to the ones observed in convalescent COVID-19 patients. A phase I dose-escalation study of 0.1 to 1.0 μg of LNP-SARS-CoV-2 S RNA is currently in progress in healthy volunteers to assess the safety of the vaccine candidate [59].

Intranasal and oral delivery of viral vector-based vaccines

In addition to conventional intramuscular vaccine administration, oral and nasal delivery approaches have been evaluated due to the potentially broad immune responses in the nasal mucosa, where SARS-CoV-2 infection occurs and the possibility to block both infection and transmission of SARS-CoV-2. In this context, intranasal administration of 1×10^{10} Ad5-S nb2 particles provided protection of macaques [29]. Moreover, Ad5 expressing the receptor binding domain (RBD) of the SARS-CoV-2 S protein elicited strong RBD-specific IgA responses and $CD4^+$ and $CD8^+$ T-cell responses with Th1-like cytokine profiles after intranasal administration in mice [60].

In another approach, a lentivirus vector expressing the full-length SARS-CoV-2 S protein was evaluated in ACE2-humanized mice [61]. Although robust immune responses were elicited, only partial protection against SARS-CoV-2 was achieved after systemic lentivirus administration. However, intranasal administration generated strong immune responses in the respiratory tract, a significant decrease in viral load in the lung, and reduced local inflammation. Studies in hamsters demonstrated prevention of deleterious lung injury. In the context of lentiviruses, although not based on intranasal administration, the LV-SMENP-DC vaccine utilizes modified DCs transduced with LV-SMENP and immunomodulatory genes [62]. In a phase I/II clinical trial volunteers will receive subcutaneously 5×10^6 lentivirus transduced DCs and 1×10^8 antigen specific CTLs for the evaluation of vaccine safety and efficacy.

Influenza virus vectors have been engineered by replacing the neuraminidase (NA) gene with a membrane-anchored form of the RBD of the SARS-CoV-2 S protein [63]. Robust neutralizing antibody responses against SARS-CoV-2, equivalent to levels observed in convalescent COVID-19 patients, were obtained after a single intranasal dose of the ΔNA(RBD)-Flu vaccine. The influenza virus-based vaccine candidate has further been subjected to registration of phase I [64] and phase II [65] clinical trials in China for the delivery as an intranasal spray.

In the context of oral delivery, adenovirus vectors have previously demonstrated good tolerability and protective immunity against influenza virus in a phase II clinical trial [66]. Oral tablets have been applied for vaccine development of an adenovirus vector expressing the full-length SARS-CoV-2 S and N proteins in combination with a Toll-like receptor-3 (TLR-3) agonist adjuvant [67]. Immunization of mice elicited strong neutralizing antibody responses, although in this case intranasal administration was preferred as the transgene expression in mice can be suppressed in the intestinal environment after oral administration [68]. However, the oral vaccine delivery approach has been evaluated in a phase I clinical trial for the VXA-COV2-1 vaccine candidate to assess safety and immunogenicity [69]. Preliminary data indicated that the vaccine was well tolerated and strong $CD8^+$ T-cell responses were induced [70]. Vaxart, the vaccine producer, claimed that the $CD8^+$ T-cell responses obtained for VXA-COV2-1 were superior to those seen for the Pfizer/BioNTech and Moderna mRNA vaccines [71].

SARS-CoV-2 variants and vaccine efficacy

Despite the success achieved for developing vaccines against COVID-19 the detection of novel SARS-CoV-2 lineages has raised concern about vaccine efficacy. For instance, the B.1.1.7 variant (alpha) was initially claimed to possess higher transmission rates and was found to spread rapidly in the UK [72]. The alpha variant carrying the N510Y mutation and deletion of amino acids 69 and 70 in the RBD of the SARS-CoV-2 S protein was determined to be 75% more transmissible than the wildtype strain with the 501N sequence [73]. Additionally, it was recently demonstrated that individuals tested positive for the alpha variant showed a

mean $\log_{10}$ viral load 1.05 higher than non-alpha variant subjects [74]. In addition to the alpha variant, the South African B.1.351 (beta) [75], the Brazilian B.1.1.28.1 (gamma) [76], and the Indian B.1.617 (delta) [77] variants have been identified. Related to vaccine efficacy, adenovirus vector-, RNA-, and protein subunit-based vaccines have been tested. A small but significant reduction in neutralizing antibody activity against the N501A and the K417N-E484K-N501Y mutations in the SARS-CoV-2 S protein was detected for the two approved RNA-based vaccines [78]. In another study, 20 volunteers vaccinated with the BNT162b2 RNA vaccine showed similar neutralizing titers to SARS-CoV-2 with either N501 or Y501 in the S protein [79]. Related to the nanoparticle encapsulated SARS-CoV-2 S protein subunit vaccine NVX-CoV22373 the efficacy against the alpha variant was 86% and against the beta variant was 60% [80]. In the case of adenovirus-based vaccines, variability related to protection efficacy has been discovered. For instance, in a phase II/III trial, similar vaccine efficacy against the alpha variant and other lineages was obtained [15]. However, reduced neutralization activity was measured against the alpha variant compared to non-alpha variants *in vitro* after ChAfdOx1 nCoV-19 vaccine administration [81]. Despite that, the vaccine provided protection against the alpha variant. However, in another study the ChAdOx1 nCoV-19 failed to provide protection against mild-to-moderated COVID-19 caused by the beta variant [82]. In contrast, the Ad26.COV2.S vaccine showed clinical efficacy against symptomatic COVID-19, also against the beta variant despite its partial resistance to neutralizing antibodies [83]. Moreover, humoral, and cellular responses against both the original SARS-CoV-2 strain and the beta variant were observed. However, the median pseudovirus neutralizing antibody titers were 5-fold lower in comparison to the original SARS-CoV-2 strain. Overall, the detected variants and potentially emerging new variants demand a thorough follow-up on vaccine efficacy and the readiness of re-engineering available vaccines to ensure the efficacy of vaccine protection.

Adenovirus vector-based vaccines and vaccine-induced immune thrombotic thrombocytopenia

Recently, rare cases of thrombocytopenia have been detected in individuals receiving COVID-19 vaccines, which have been referred as VITT [84]. Although only a few hundred cases have been reported among the more than 150 million vaccinated people worldwide, the issue must be addressed properly. After the first detected cases in individuals vaccinated with the ChAdOx1 nCoV-2 vaccine, persons vaccinated with the Ad26.COV2.S vaccine also developed VITT [85]. As adenovirus gene transfer had previously been associated with VITT it was thought to have been induced by adenovirus administration [86]. However, not before long, cases of VITT were identified after vaccinations with the BNT162b2 and mRNA-1273 RNA vaccines [87]. One of the common factures for all vaccines causing VITT is the utilization of the SARS-CoV-2 S protein as the antigen. In a recent study, it was described that transcription of wildtype and codon-optimized SARS-CoV-2 S enables alternative splicing, which leads to production of C-terminal truncated soluble S protein variants [88]. It was postulated that the generated soluble S protein variants are responsible for severe side effects by binding to ACE2 expressing endothelial cells in blood vessels leading to thromboembolic events. The disease mechanism was termed Vaccine-Induced COVID-19 Mimicry (VIC19M). Other hypotheses for the cause of VITT include the interaction of the SARS-CoV-2 S protein with C-type lectin receptors, heparin sulfate proteoglycans and the CD147 receptor, and interaction of the adenovirus vector with the CD46 receptor or platelet factor 4 antibodies [89]. Although some ideas and hypotheses have been presented, the reasons for causing VITT are still unresolved and requires further investigation.

Conclusion

Application of viral vectors for the development of COVID-19 has been extremely successful leading to EUA for several adenovirus-based vaccines in roughly one year since the onset of the pandemic. Although providing slightly lower vaccine efficacy than seen for RNA-based vaccines, they have been commonly used in mass vaccinations. However, comparison of efficacy of different COVID-19 vaccines is complicated. Although phase III clinical trials involve a large number of vaccinated individuals, the different vaccines are generally evaluated at different geographical locations and at different phases of the pandemic, and not compared in parallel. In this context, the adenovirus-based ChAdOx1 nCoV-19 and the mRNA-based BNT162b2 and mRMA-1273 vaccines were originally evaluated before the SARS-CoV-2 alpha, beta, gamma, and delta variants

emerged, which does not make the comparison to vaccines developed more recently accurate. However, several clinical trials are in progress for the current COVID-19 vaccines to determine vaccine efficacy against the latest SARS-CoV-2 variants.

One advantage of adenovirus-based vaccines compared to RNA-based vaccines is the superior storage and transportation requirements as these vaccines can be kept at 4°C and for shorter time at room temperature, whereas RNA-based vaccines require storage at -20°C (Moderna) and -80°C (Pfizer/BioNTech). Moreover, the Ad26.COV2.S has demonstrated efficacy after a single dose administration compared to the other vaccines requiring a prime-boost regimen. In addition to adenoviruses, vaccine candidates based on vectors of MV, vaccinia virus, rhabdoviruses, lentiviruses and influenza viruses have already been subjected to clinical trials. An interesting approach has been to employ self-amplifying RNA viruses for liposome nanoparticle-based delivery of mRNA. In this context, the VEE-based LNP-RNA vaccine candidate is currently under phase I clinical evaluation.

Although the current vaccines have demonstrated efficacy in humans, the occurrence of novel SARS-CoV-2 variants with enhanced transmissibility and pathogenicity have increased the demand on vaccine efficacy and the need for preparedness of re-engineering capacity of existent vaccines. Moreover, the findings of rare cases of VITT in individuals receiving COVID-19 vaccines require thorough investigations into the cause to be able to guarantee the highest possible safety for vaccine administration to prevent the spread of SARS-CoV-2 and to eradicate the pandemic.

Abbreviations

AAV: adeno-associated virus

ACE2: angiotensin-converting enzyme 2

ChAdV68: chimpanzee adenovirus serotype 68

COVID-19: coronavirus disease 2019

CTLs: cytotoxic T cell lymphocytes

DCs: dendritic cells

EUA: Emergency Use Authorization

FDA: Food and Drug Administration

GRAd: gorilla adenovirus

Ig: immunoglobulin

i.n.: intranasal

LNP: lipid nanoparticle

LV: lentivirus

LV-SMENP: lentivirus vector expressing severe acute respiratory syndrome coronavirus 2 minigene

MV: measles virus

MVA: modified vaccinia virus Ankara

N: nucleocapsid

NDV: Newcastle disease virus

RBD: receptor binding domain

S: spike

SAM: self-amplifying mRNA

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Th1: T helper 1

VEE: Venezuelan equine encephalitis virus

VITT: vaccine-induced immune thrombotic thrombocytopenia

VSV: vesicular stomatitis virus

VSV-ΔG: vesicular stomatitis virus G protein

Declarations

Author contributions

The author contributed solely to the work.

Conflicts of interest

The author declares that he has no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

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Not applicable.

Availability of data and materials

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