Vaccines And Advanced Vaccines: -A landscape for advanced vaccine technology against infectious disease ,COVID-19 and tumor

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Abstract

The vaccine helps to provoke the immune system and is an efficacious means for disease prevention and treatment. At this particular time of the COVID-19 outbreak, the vaccine for COVID-19 is urgently needed to save tens of thousands of people' s lives. Here we give some basic information on vaccine classification, generation, and application, and make a brief review on the current status of COVID-19 vaccine and tumor vaccine development both in the clinical trial stage and pre-clinical stage.

Keywords

COVID-19, vaccine, COVID-19 vaccine, AAV vector based vaccine, vector-based vaccine, Adenoviral vector-based vaccines, lentiviral vector-based vaccines, DNA vaccine; RNA vaccine, tumor vaccine,

1. Landscape of vaccines

A vaccine is any substance produced from various pathogenic microorganisms and can spur the body's immune system to generate antibodies for the prevention, diagnosis, or treatment of diseases when given to the body, including artificial active immunity vaccine, artificial passive immunity vaccine and novel vaccine (Fig. 1). Traditionally, the artificial active immunity vaccines can be divided into three categories: dead vaccines, live vaccines, and toxoid (Fig. 1). Dead vaccines are dead parts or wholes of germ cells, once injected into people or animals, they can trigger mild immune responses, containing pertussis vaccine, typhoid vaccine, meningococcal vaccine, cholera vaccine, etc.; while live vaccines are living parts or all of germ cells, most of them are attenuated to reduce the risk but also provide immunity for the body, such as BCG vaccine, polio vaccine, measles vaccine, plague vaccine, etc.; toxoid is a kind of extracellular toxin treated by formaldehyde, which loses the toxicity but retains the immunogenicity, such as diphtheria toxoid, tetanus toxoid. Considering the long-term required for immune system activation using artificial active immunity, the artificial passive immunity vaccine is designed for rapid disease treatment or emergency prevention, including antitoxin, immunoglobulin, cytokine, and monoclonal antibodies.

Besides these two kinds of immunity vaccines, some novel vaccines have been developed or created, such as subunit vaccine, conjugate vaccine, synthetic peptide vaccine, and genetic engineering vaccine (recombinant protein-based vaccines, lipid-based vaccines, polysaccharides-based vaccines, viral vector-based vaccines, and mRNA/DNA vaccines, etc.). However, some of them, such as lipid-based vaccines and polysaccharides-based vaccines, only result in poor immunogenicity. The reasons might be that some antigen structures with adjuvant activity are discarded during vaccine preparation, or some vaccines can only trigger one kind of immune responses, such as cell-mediated or humoral immune responses. Among them, viral vector-based vaccines, and mRNA/DNA vaccines can provoke both humoral and cell-mediated immune responses, which are promising candidates with great potential in the future.

Figure 1. Classification of Vaccines.

2. Viral vector-based vaccine

Viral vector-based vaccines are vaccines that can deliver specific antigen gene to target cells based on the infection ability of viruses, produce antigens via the nutrition substances in host cells, and then provoke immune responses with the newly synthesized antigens. Compared with the traditional vaccines, viral vector vaccines have a great number of advantages: ① highly efficient in gene transduction; ② mediate specific gene delivery to target cells; ③ induce of both humoral and cell-mediated immune responses; ④ better efficacy and safety; ⑤ just need low administration dose; ⑥ easy to be applied into large-scale manufacturing; ⑦ possessing widespread potential target diseases, ranging from infectious diseases to cancers. As well, some drawbacks also have been discovered: ① several kinds of vectors mediate gene integration into host genome, which may lead to cancer; ② some hosts may be exposed to antigens prior to the vaccine administration, which may result in the production of neutralizing antibodies (pre-existing immunity) and thus reduce the vaccine efficacy [1].

To date, numerous kinds of viral vectors have been introduced to produce vaccines, such as adeno-associated virus (AAV) vectors, adenoviral vectors and lentiviral vectors [2]. Different kinds of viral vectors have their advantages and drawbacks, which are summarized in Table 1.

Table 1 - Comparison between Lentiviral, Adenoviral and AAV vectors.			
Viral vectors	Lentivirus	Adenovirus	AAV
Genome	ss RNA	ds DNA	ss DNA
Integration	Yes	No	No
Packaging capacity	4kb	5.5kb	2kb

Time to peak expression	72h	36h-72h	cell: 7 days; animals: 2 weeks
Sustainable time	Stable expression	Transient expression	> 6 months
Cell Type	Most dividing/non-dividing Cells	Most dividing/non-dividing Cells	Most dividing/non-dividing Cells
Titeration	10 ⁸ TU/ml	10 ¹¹ PFU/ml	10 ¹² v.g./ml
Animal experiment	Low efficiency	Lowest efficiency	Most suitable
Immune Response	Medium	High	Mild

1) AAV vector-based vaccines

Adeno-associated virus (AAV) is a small single strand DNA virus, member of human parvovirus [3, 4], approximately 25nm in diameter and encapsidates a single-stranded DNA genome of 4.7 kilobases (Fig. 2A). The genome consists of two large open reading frames (ORFs) flanked by 145bp inverted terminal repeats (ITR), which are the only cis-acting elements required for AAV genome replication and AAV packaging. The left ORF encodes four replication proteins, Rep40, Rep52, Rep68, and Rep78, in charge of site-specific integration, as well as regulation of AAV capsid formation initiation within the AAV genome, while the right ORF encodes the viral structural proteins, VP1, VP2, and VP3, which interact together to assemble into icosahedral virion shells comprising 60 subunits each (Fig. 2B).

Figure 2. AAV genome map. (A) The capsid structure of AAV. (B) The positions of the three promoters (p5, p19, p40) as well as the seven protein coding regions of the AAV have been highlighted.

Principle

Principle of AAV entry into cells

AAV transduces cells through several stages: ① viral binding to cell surface receptor/coreceptor, ② endocytosis of the virus, ③ intracellular trafficking of the virus through the endosomal compartment, ④ endosomal escape of the virus, ⑤ intracellular trafficking of the virus to the nucleus and nuclear import, ⑥ virion

uncoating, ⑦ viral genome conversion from a single-stranded to a double-stranded genome capable of expressing an encoded gene [5-7]. Since AAV has no ability to encode polymerases, AAV is dependent upon cellular polymerase activity to replicate its own genome [8]. The presence of a helper virus such as adenovirus is indispensable for wild-type AAV to facilitate gene expression and replication (Fig. 3A). Without helper virus, expression of Rep68/Rep78 would be restricted owing to Ying Yang 1 (YY1) repression of the P5 promoter, leading to inhibition of AAV genome replication and gene expression, and initiation of AAV chromosome integration (Fig. 3B) [9]. AAV establishes latency by undergoing specifically integration into a genome site, termed as the adeno-associated virus integration site 1 (AAVS1), a 4kb region on chromosome 19 (q13.4).

Figure 3. AAV life cycle. (A) AAV can amplify itself with the help of helper virus. (B) AAV establishes latency by undergoing specifically integration into AAVS1 without helper virus.

Immune responses induced by AAV-based vaccine

During the entry of AAV into host cells, AAV virions may uncoat and release their genomes into the endosome, and be recognized by toll like receptor 9 (TLR9) of plasmacytoid dendritic cell (pDC) to provoke innate immune response and produce interferon (IFN) α/β [10]. This process is dependent on MyD88 signaling, but not the form of transgene or capsid serotype [10]. Besides TLR9, TLR2 dependent cytokine expression was also observed in Kupffer cells [11]. Moreover, some AAV virions are degraded and processed into peptides within proteasomes, and then presented by MHC I of antigen presenting cells (APCs), such as conventional dendritic cells (cDCs), which can be targeted by capsid-specific CD8⁺ T cells to lyse virally infected cells [12]. In addition to IFN α/β , CD40-CD40L co-stimulation by CD4⁺ T helper cells, is required for cross-priming of CD8⁺ T cells against AAV capsid [13]. CD4⁺ T helper cells are also indispensable to generate memory responses and stimulate B cells to produce antibody against AAV capsid, which is dependent on

MyD88 signaling [14, 15].

Application

AAV serotypes

Over the past decades, numerous AAV serotypes have been identified with variable tropism. To date, 12 AAV serotypes and over 100 AAV variants have been isolated from adenovirus stocks or from human/nonhuman primate tissues. Among them, AAV2, AAV3, AAV5, AAV6 were discovered in human cells, while AAV1, AAV4, AAV7, AAV8, AAV9, AAV10 (AAVrh10), AAV11, AAV12 in nonhuman primate samples [16]. Different serotypes have different tissue tropism, which are summarized in Table 2.

Table 2 — AAV serotypes and their respective Tropism.								
AAV Serotype	Tissue tropism							
	CNS	Retina	Lung	Liver	Pancreas	Kidney	Heart	Muscle
AAV1	√	√			√		√	√
AAV2		√		√		√		
AAV3		√	√	√			√	
AAV4	√	√					√	
AAV5	√	√	√		√			
AAV6	√		√	√			√	√
AAV7				√				√
AAV8		√		√	√			√
AAV9	√		√	√			√	√
DJ		√	√	√		√		
DJ/8		√		√				√
Rh10	√		√	√			√	√
AAV-retro	√	√						√
AAV-PHP.B	√						√	√
AAV8-PHP.eB	√							√
AAV-PHP.S	√						√	√

Recombinant AAV

Though wild-type AAV is not associated with human disease, it is naturally defective and requiring helper adenovirus or herpes simplex virus (HSV) coinfection for AAV replication, so recombinant AAV (rAAV) has been developed for gene therapy or vaccines by replacing the viral genome with gene of interest (GOI) to reduce the risk. Traditionally, rAAV vectors used in clinical trials were prepared with a plasmid containing the therapeutic gene flanked by AAV-inverted terminal repeats (ITRs), co-transfected with AAV packaging plasmid pAAV-RC (AAV replication and AAV

capsid) and pHelper (AAV helper plasmid) (Fig. 4). The adenovirus helper factors, such as E1A, E1B, E2A, E4 ORF6 and VA RNAs, would be provided by the third helper plasmid. Due to the deletion of Rep and Cap coding regions between the ITRs, rAAV vectors cannot integrate into the genome of host cells, just persist in an episomal form, which significantly reduced their tumorigenicity.

Figure 4. The three plasmids co-transfection system of recombinant AAV. pAAV-GOI: an AAV ITR-containing plasmid carrying the gene of interest (GOI); pAAV-RC: an AAV serotype plasmid that carries Rep and Capsid genes; pHelper: an AAV helper plasmid that provides the helper genes isolated from adenovirus.

AAV-based gene therapy

To date, more than 244 clinical trials have been carried out using AAV vectors for gene delivery [17], and promising gene therapy outcomes have been achieved from Phase 1, Phase 2 and Phase 3 trials for a great number of diseases, including lipoprotein lipase deficiency (LPLD) [18], spinal muscular atrophy (SMA) [19], retinal dystrophy [20, 21], cystic fibrosis [22, 23], Duchenne Muscular Dystrophy [24], Hemophilia [25], congestive heart failure [26], Parkinson's disease [27] and Rheumatoid Arthritis [28, 29].

AAV-based vaccine development

But, as a viral vector used for vaccine production, AAV only induces mild immune responses, which is not enough for vaccine to provoke the immune system in host. Several animal studies show that AAV vector-based vaccines can be used to defense HIV-1 [30-32], influenza [33], and papillomavirus [34] and have great potentials in clinical applications. However, AAV vector-based vaccines are rarely applied in clinical trials. Some of the examples are listed in the following Table 3. There are two reasons: ① AAV vectors only cause mild humoral and cellular immunity; ② infectious vaccines transduce a large population of people ranging from children and adolescents, and more safety risks need to be considered. Therefore, compared to the gene therapy with AAV vectors, there is a long way for the clinical applications of AAV vector-based vaccines.

<p>Table 3 - Examples of AAV vector-based vaccines</p>
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Disease	Vaccine component	Status	Clinical trials
HIV	AAV2	Phase I	NCT00482027
HIV	AAV2	Phase II	NCT00888446
HIV	AAV8	Phase I	NCT03374202
HIV	AAV1	Phase I	NCT01937455
Stage IV gastric cancer	AAV-DC-CTL	Phase I	NCT01637805
Stage IV gastric cancer	AAV-DC-CTL	Phase I	NCT02496273

Advantages and disadvantage

AAV viral vector has been developed into a very attractive candidate for gene delivery due to various advantages: ① superior biosafety rating of recombinant AAV after removing most AAV genome elements; ② stable physical properties; ③ broad range of infectivity, AAV has the ability to infect both dividing and quiescent cells in vivo; ④ mediate long term and stable gene expression.

However, there are also some drawbacks for AAV to be used as vaccine vector: ① limited cloning capacity (less than 4.7kb) of the vector, which restricts its use in gene delivery of large genes [35]; ② only inducing mild immunity, restraining the vaccine development; ③ pre-existing immunity and neutralizing antibodies (NAB) against AAV vectors may attenuate the effect of AAV-based gene therapy or vaccines [36].

Optimization strategies

To improve the efficacy of AAV vector for vaccine development, several strategies are adopted: ① assemble and recombine proteins between different viruses, which can produce hybrid rAAVs, such as transcapsidation, which is a process involving the packaging of the ITR from one AAV serotype into the capsid of another serotype, which may determine the tissue tropism of hybrids. ② Recombine, redesign, or introduce random mutations into the capsid protein of AAV by different methods to artificially increase the variance of AAV serotypes, and then screen the appropriate AAV serotypes, including rational design AAV capsid [37], AAV directed evolution [38], point mutation [39], peptide display [40], and DNA shuffling [41]. ③ In

combination with other kinds of vaccines.

GeneMedi holds the expertise at AAV production, you can find more information and protocols about AAV on this website: <https://www.genemedi.net/i/aav-packaging>.

2) Adenoviral vector-based vaccines

Adenovirus (AdV) is a member of the family Adenoviridae, whose name derives from their initial isolation from human adenoids in 1953 [42]. It is a medium sized (90-100nm) and non-enveloped virus with an icosahedral nucleocapsid containing a 36kb double stranded DNA genome (Fig. 5A). Hexon and penton structures form the capsid of AdV, and fiber protein mediates the binding of the virion to the cell surface and is a major determinant of viral tropism. Adenovirus transcription is a two-phase event, early and late, occurring before and after viral DNA replication, respectively (Figure. 5B). The early transcribed regions are E1A, E1B, E2A, E2B, E3, and E4, involved in viral transcription regulation, viral DNA replication, and the suppression of host immune response during infection. The late transcribed genes are L1-L5, encoding viral capsid components (Fig. 5B).

Figure 5. Schematic of the adenovirus genome and adenovirus-based vectors. (A) Capsid crystal structure. (B) Adenovirus gene map. Top panel: a simplified map of the adenovirus serotype 5 genome showing the early genes (E1–E4) and the region from which the major late transcript is produced. Middle panel: general structure of an early region 1 (E1)-deleted Ad vector. Bottom panel: general structure of a helper-dependent Ad vector [43].

Principle

Principle of AdV entry into cells

For most serotypes, adenovirus infection is mediated by the high-affinity binding of the fiber-knob region to a receptor of target cell, named as the coxsackie-Ad receptor (CAR), which mainly determines the viral tropism [44]. Upon attachment, interaction between the penton-base Arg-Gly-Asp (RGD) and cellular αv integrins, which can stimulate actin polymerization, leads to internalisation of the virus into the endosome. Then the endosome acidifies, resulting in disassociation of capsid proteins and transportation of viral DNA into nucleus. Without integration into host genome, adenovirus genome remains in an episomal state, which guarantees the low risk

of mutation (Fig. 6). Life cycle of adenovirus is separated by DNA replication process into two distinct phases: the early and late, occurring before and after viral DNA replication, respectively. After the synthesis of viral genome and capsid, they are assembled into viral products, releasing out of cell, and the infected cell starts lysis [45]. To prevent infected cell lysis, recombinant replication deficiency virus has been developed as a gene delivery tool to replace wild-type adenovirus (recombinant AdV in “application” part). Once packaged into a E1-complementing cell line, which provides the E1 products in trans, such as QBI 293A Cells, recombinant viral will be easily propagated.

Figure 6. Infection process of adenovirus. CAR receptor-fiber-knob adenovirus interaction and internalization process [46].

Immune responses induced by AdV

The entry of AdV into human body stimulates a wide spectrum of innate cellular responses, which might last a few minutes to hours and result in blood pressure changes, thrombocytopenia, inflammation, and fever [47]. AdV vector in blood activates vascular endothelial cells to release von Willebrand factor (vWF), induces platelets to expose the adhesion molecule P-selectin, and promotes the formation of platelet-leukocyte, ultimately leading to thrombocytopenia and bleeding [48]. Additionally, the hexon component of AdV capsid can bind to coagulation factor X (FX) to activate TLR4 on the surface of splenic macrophages and thereby stimulate NF- κ B dependent activation of IL-1 β , which may help recruit polymorphonuclear leukocytes to the marginal zone of the spleen and clear virus from the spleen rapidly [49, 50]. Besides binding to coagulation proteins, AdV in blood vessel can also bind to component C3 and natural [51-55]. Antibody-AdV complexes can provoke inflammatory cytokine and chemokine responses in macrophages via the intracellular antibody receptor TRIM21 [56-59].

In addition to innate immune responses in circulation, AdV virions can also be trapped by splenic macrophages (MFs) of the MARCO subset via the binding of fiber knob of the capsid to integrin b3 receptor [60]. This binding process leads to the release of IL-1 α and activation of IL-1 receptor, promoting chemokines production and attracting other innate immune cells to kill AdV infected MFs [61]. AdV also activates the NLRP3 inflammasome to recruit proinflammatory caspases and promote

IL-1 β expression, thereby resulting in necrotic cell death [62]. Cytosolic sensing of AdV DNA via cyclic GMP-AMP synthase (cGAS) binds to stimulator of interferon genes (STING) to promote IRF3 phosphorylation to produce type I IFN [63, 64]. Moreover, AdV DNA is also sensed by the endosomal receptor TLR3, TLR7, and TLR9 to activate MyD88 signaling pathway. Nuclear sensing mechanisms of AdV DNA can also promote or inhibit immunity [65, 66]. These pathways cooperate to regulate the immune responses triggered by AdV vectors.

In the meantime, AdV also provoke highly effective adaptive immune responses, and the mechanism is similar to that of AAV. But contrary to AAV, AdV triggers a particularly strong CD8⁺ T cell responses, which is facilitated by potent induction of Th1 immunity [67]. Due to the induction of extremely strong immune responses, AdV vectors are a prerequisite vector for vaccine development.

Application

Recombinant AdV

Since wild-type adenovirus is associated with a wide range of illnesses and enlists a variety of immune responses, so recombinant replication deficiency virus has been an attractive vector for gene therapy [68]. To date, there have been many different generations of adenovirus vectors, differing in the extent to which the genome from wild-type adenovirus is attenuated. Based on human adenovirus type 5 (Ad5), recombinant adenovirus (Ad) a replication-defective adenoviral vector system, is widely used for gene delivery in most dividing and non-dividing cells based on its advantages in high transduction efficiency, high level of transgene expression, and broad range of viral tropism [69]. Traditionally, recombinant adenovirus vectors used in gene delivery were prepared with a plasmid containing the transgene flanked by inverted terminal repeats (ITRs), co-transfected with packaging plasmid pAd-BHGlox(delta)E1,3 (Fig. 7). Once packaged into a E1-complementing cell line, such as QBI 293A cells, recombinant viral will be easily propagated.

Figure 7. The two plasmids co-transfection system of recombinant adenovirus. pAd-EF1-MCS-CMV-EGFP: an adenovirus ITR-containing plasmid carrying multiple clone sites, which can be cloned into a transgene; pAd-BHGlox(delta)E1,3: a packaging plasmid.

AdV vector-based gene therapy

To date, more than 535 clinical trials have been carried out using adenovirus vectors for gene delivery [17], and promising gene therapy outcomes from recombinant adenovirus have been achieved from clinical trials for a great number of diseases, especially for cancer treatment, such as prostate cancer [70], chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [71], non-small cell lung cancer (NSCLC) [72], melanoma [73], renal cell carcinoma [74].

AdV vector-based vaccine development

As a viral vector used for vaccine production, AdV induces strong immune responses and show better superiority than other viral vectors. In clinical trials, AdV vector-based vaccines have been used to prevent HIV-1 [75, 76], influenza [77], tuberculosis [78], and solid tumors [79]. Some of examples in clinical trials are listed in Table 4 [80]. However, there are also some reports with discouraging outcomes. For example, Ad5 vector with neutralizing antiserum against HIV contrarily significantly facilitated HIV infection besides the enhanced immune responses [81]. The pre-existing anti-AdV immunity against Ad5 is proved to be another limiting factor for the clinical application of Ad vector-based vaccines [82]. Therefore, although AdV vector exhibits some advantages over AAV vector in vaccine production, there are also some safety hazards and pre-existing anti-AdV immune responses needed for further exploration.

Table 4 - Examples of AdV vector-based vaccines [80]			
Target	Disease	Status	Clinical trials
Infectious diseases	HIV	Phase I/II	NCT00865566 ; NCT00413725
	HIV	Phase II	NCT00415649 ; NCT00125970 ; NCT00498056 ; NCT00123968 ; NCT00095576 ; NCT00080106 ; NCT00350623 ; NCT01159990
	Malaria	Phase I/II	NCT00870987 ; NCT00392015 ; NCT01366534 ; NCT01397227 ;
	Malaria	Phase II	NCT01666925 ; NCT01658696 ; NCT00890760
	Hepatitis C virus	Phase I/II	NCT02309086

	Ebola virus	Phase I/II	NCT02289027
	Ebola virus	Phase II	NCT02344407
	Tuberculosis	Phase I/II	NCT01017536
	Tuberculosis	Phase II	NCT01198366
	Rotavirus	Phase III	NCT01305109
Cancer	Colon/breast/lung/head/neck/renal	Phase I/II	NCT00049218 ; NCT00669136 ; NCT00880464 ; NCT00776295 ; NCT00082641 ; NCT00093548 ; NCT01042535 ; NCT00617409 ;
	Colon/breast/lung/head/neck/renal	Phase II	NCT00589186 ; NCT01147965 ; NCT01924156
	Prostate	Phase II	NCT00583752 ; NCT00583024
	Lymphoma	Phase II	NCT00849524 ; NCT00942409
	Solid tumor/tissue sarcoma	Phase I/II	NCT02285816 ; NCT01898663
	Leukemia/glioblastoma	Phase II	NCT01773395 ; NCT00589875
	Melanoma	Phase I/II	NCT00039325 ; NCT00704938 ; NCT00010309
	Melanoma	Phase II	NCT00004025

Advantages and disadvantage

There are many advantages of AdV as a vector for clinical trials: ① well tolerated with no obvious influences on the cell viability after infection; ② great packaging capacity (up to 8kb); ③ broad range of infectivity, from dividing cells to non-dividing cells; ④ high infection efficiency; ⑤ no integration ability into the host genome, remaining epichromosomal in host cells, thus no oncogenicity; ⑥ inducing a wide variety of immune responses, including humoral and cellular immunity. These advantages make AdV as an excellent vector for vaccine development.

Although adenovirus benefits a great deal of disease prevention, it does present some

drawbacks: ① AdV-mediated gene delivery may not sustain for long time, just transient expression; ② pre-existing immunity and neutralizing antibodies (NAB) against AdV vectors might attenuate the preventive effects of AdV vector-based vaccines [83]; ③ despite reduced overall virulence, recombinant Ad5-based vectors exhibit a strong tropism for liver parenchymal cells due to the high expression level of CAR in hepatocytes, which increases hepatotoxicity and limits the clinical use of AdV vectors [84].

Optimization

Several measures were taken to improve the efficacy of AdV vector for vaccine development: ① modify the fiber protein to alter the tissue tropism and decrease the hepatic toxicity [85]; ② reduce viral vector-derived immune responses by changing virus gene expression, such as deleting E1 and E4 gene of AdV [86]; ③ modify the fiber protein to enhance the transduction of T cells and dendritic cells [87].

Genemedi got a rich experience in adenovirus packaging, you could find more information on <https://www.genemedi.net/i/adenovirus-packaging>.

3) Lentiviral vector-based vaccines

Lentivirus (*lente*-, Latin for "slow") is a genus of retroviruses, medium sized (80-100nm) and enveloped, slightly pleomorphic, spherical with an isometric nucleocapsid containing two copies of positive-sense ssRNA genome (Fig. 8A). Most lentiviral vectors are based on the Human Immunodeficiency Virus (HIV), which causes AIDS, a chronic and deadly diseases in human or other mammalian species [88]. Its DNA genome, transcribed from HIV-1 ssRNA, is approximately 9.7 kb and contains 9 open reading frames (ORFs), which are flanked by 5' and 3' long terminal repeats (LTRs), which are required for HIV-1 life cycle, such as reverse transcription, integration, and gene expression (Fig. 8B).

Figure 8. Schematic of the lentivirus genome and lentivirus-based vectors. (A) Capsid crystal structure. (B) The viral genome encodes structural, regulatory and accessory genes flanked by LTRs [89]. There are 9 ORFs in lentiviral genome: *env* and *gag* responsible for structural proteins; *pol* for reverse transcriptase and integrase; *tat* and *rev* for gene regulatory proteins; *vif*, *vpr*, *vpu*, and *nef* for viral accessory proteins.

Principle

Principle of lentivirus entry into cells

HIV-1 virus enters host cells through binding to the CD4 receptor or a coreceptor (CCR5 or CXCR4) with gp120 protein, thereby anchoring itself onto host cell surface, allowing fusion between the cellular and viral membranes. After entry into the cell, the viral nucleoprotein together with the contents, i.e. the genomic ssRNA, is released into cytoplasm. Then, utilizing the cellular nucleotides as the building blocks, double-stranded DNA is generated from the virus genome ssRNA directed by the HIV-1 reverse transcriptase (RT) in a nucleoprotein complex termed the RTC. Together with other viral proteins, the newly synthesized DNA constitutes an integration-competent nucleoprotein complex, migrating into host cell nucleus and mediating integration of viral DNA into host chromatin. Integrated viral DNA, named as provirus, becomes part of host genome and serves as a transcription template for the synthesis of viral mRNA and genomic RNA. Following the synthesis of viral genomes and proteins, the viral components are assembled together to produce new virions, the virus particles then bud out of host cell and undergo a maturation step to generate infectious HIV-1 (Fig. 9) [90].

Figure 9. Schematic representation of lentivirus replication [90].

Immune responses induced by lentivirus

Compared to HIV-1 virus, lentiviral vectors elicit weaker IFN α responses from pDC. SsRNA molecules of lentiviral vector in endosomes can be sensed by TLR7, while the reverse-transcribed CpG DNA is sensed by TLR9. TLR7 and TLR9 both contribute to induction of T1 IFN [91, 92] and regulation by mTOR pathway [93]. In addition to innate immune responses, lentiviral vector efficiently transduces APCs, such as MFs

and DCs, which significantly facilitates the immune responses against transgene antigen. It was reported that transgene antigen expression in pDCs mainly drives immune responses, while antigen expression in myeloid cells may not obviously provoke immune responses [94, 95]. Furthermore, cell-derived MHC I molecules also helps trigger T cellular immune responses.

Application

Recombinant lentiviral vector

Since wild-type HIV-1-based lentivirus is associated with destruction of host immune system, especially CD4⁺ helper T lymphocytes, multiple generations of lentivirus vectors have been designed with enhanced safety features and as attractive vectors for gene therapy and vaccine production. To date, there have been several generations of HIV-1-based lentivirus vectors by deleting the HIV viral envelope and some of the regulatory genes not required during vector production [89]. The recombinant lentiviral vectors in GeneMedi are prepared based on three plasmids co-transfection system: vector plasmid pLV-CMV-MCS-T2A-PURO, envelope expressing plasmid pMD2G, and packaging plasmid pSPAX2 (Fig. 10). Once packaged into 293T, recombinant lentiviral vectors will be easily produced.

Figure 10. The three plasmids co-transfection system of recombinant lentivirus. pLV-CMV-MCS-T2A-PURO: a lentivirus LTR-containing plasmid carrying multiple clone sites, which can be cloned into a transgene; pMD2G: envelope VSVG expressing plasmid; pSPAX2: packaging plasmid, containing sequences of *gag*, *pol*, *tat*, and *rev*.

Lentiviral vector-based gene therapy

To date, more than 236 clinical trials have been carried out using lentivirus vectors for gene delivery [17]. For instance, lentiviral vector-based gene delivery into CD34⁺ HSCs has been used as an alternative in clinical trials and proved to be effective in treatment of several diseases [96], including β -thalassemia [97], X-linked adrenoleukodystrophy (ALD) [98], metachromatic leukodystrophy [99, 100], and Wiskott-Aldrich Syndrome [101].

Lentiviral vector-based vaccine development

In addition, as a viral vector used for vaccine production, lentiviral vectors can transduce antigen-presenting cells with high efficiency, such as dendritic cells [102], which provides some priority to lentiviral vector-based vaccines. Animal studies demonstrate that lentiviral vector-based vaccines can provoke both CD8⁺ T-cells and CD4⁺ T-cell responses and show great defense effects against melanoma by targeting NY-ESO-1 antigen [102, 103], or Melan-A/MART-1 antigen [104]. Besides, lentiviral vector-based HIV vaccines induce Gag-specific T-cell responses [105-107]. Some examples of LV vector-based vaccines are listed in the following Table 5. Although promising outcomes by lentiviral vector-based have been achieved in animal studies, the virulence of lentivirus still raises safety concerns. As the virulence of immunodeficiency virus is species-specific, feline immunodeficiency virus (FIV)-based vectors have been developed [108] to combat HIV-1 and the herpes simplex virus (HSV) with great potential [109, 110].

Table 5 - Examples of LV vector-based vaccines			
Disease	Vaccine component	Status	Clinical trials
Leukemia	LV-mediated genetically engineered AML cells	Phase I	NCT00718250
ALL	LV-mediated IL-12 expression in AML cells	Phase I	NCT02483312
CD19 ⁺ B cell Leukemia and Lymphoma	ARTEMIS™	Early Phase 1	NCT03895944

Advantages and disadvantages

Recombinant lentiviral vectors have a lot of advantages for vaccine development: ① mediating long-term and stable exogenous gene expression by integrating into the host genome; ② great packaging capacity; ③ highly efficient in transfection in both dividing cells to non-dividing cells; ④ low pre-existing immunity. However, lentiviral-mediated gene integration into host genome, which might arouse the risk of tumorigenesis. Additionally, the virulence of lentiviral-derived backbone, i.e. HIV-1, also arouses serious safety concern.

Optimization

To avoid the virulence of HIV-1, FIV-based viral vectors [108] were discovered and showed great potential for the prevention of HIV-1 and HSV [109, 110]. Moreover,

integration-deficient LV vectors are being exploited for vaccine development [111, 112].

4) Comparison of commonly used viral vector-based vaccines

Viral vector-based vaccines can provoke innate and adaptative immune responses in host system besides the transgene antigens, which makes them preferred during vaccine design and development. Nevertheless, the immune responses induced by different viral vectors may have some differences. In brief, lentiviral vectors efficiently transduce antigen-presenting cells, but mediate exogenous gene integration into host genome, showing promising prospects as vaccine vectors, especially for the prevention of hematopoietic diseases. Adenoviral vectors induce high immune responses but have pre-existing immunity, exhibiting great promise in vaccine generation and gene therapy against cancers. Whereas, AAV vectors, just inducing mild immune responses and having a large number of serotypes, are the most excellent gene therapy vector but not very suitable to be vaccine vectors (Table 6).

Table 6 - Comparison among viral vector-based vaccines			
Vector vaccines	Lentiviral vector-based vaccines	Adenoviral vector-based vaccines	AAV vector-based vaccines
Immune responses	Medium	Highest	Mild
Advantages	① mediating long-term and stable exogenous gene expression by integrating into the host genome; ② great packaging capacity; ③ highly efficient in transfection in both dividing cells to non-dividing cells; ④ low pre-existing immunity	① well tolerated with no obvious influences on the cell viability after infection; ② great packaging capacity (up to 8kb); ③ broad range of infectivity; ④ high infection efficiency; ⑤ no integration ability into the host genome; ⑥ inducing a wide variety of immune responses.	① superior biosafety rating; ② stable physical properties; ③ broad range of infectivity; ④ mediate long term and stable antigen gene expression.

Drawbacks	① some risk of tumorigenesis; ② the virulence of vector backbone may raise some safety concern	① inducing transient expression of antigen; ② pre-existing immunity and neutralizing antibodies (NAB); ③ recombinant Ad5-based vectors may have hepatotoxicity	① limited cloning capacity ; ② only inducing mild immunity; ③ pre-existing immunity and neutralizing antibodies (NAB)
Packaging capacity	Medium	Highest	Lowest
Suitable for vaccine	Suitable	Most suitable	Not suitable

GeneMedi provides professional services on viral packaging, you could find more information on the following website: <https://www.genemedi.net/>.

3. DNA based vaccines

Traditionally, vaccines are prepared with killed or attenuated viruses or bacteria, which might have serious security issues for the development of HIV vaccines. Additionally, vector-based vaccines may induce anti-vector immunity, which might interfere the immune responses provoked by vaccines [113]. Thus, DNA vaccines were first created in 1990 with finding that delivery of recombinant plasmid may allow the expression of exogenous antigen protein [114]. Then, the immune responses [115] and protection against lethal influenza by exogenous plasmid DNA were also discovered [116, 117]. Following these studies, DNA vaccines are demonstrated to be effective for the various diseases, such as infectious diseases, cancers, autoimmune diseases, and allergic diseases.

Generally, the optimized antigen DNA and molecular adjuvant are cloned into plasmid backbone, named as recombinant plasmid. After amplification and purification of the recombinant plasmids, they are delivered into host cells. Utilizing the nutrients and materials of host cells, antigens are transcribed, expressed, and assembled. On the one hand, antigen peptides are recognized and presented on major histocompatibility complex (MHC) class I, while secreted antigen proteins are captured and processed by antigen-presenting cells (APCs) and then presented by MHC class II (MHC II). APCs carrying MHC-antigen peptide complex migrate to

lymph node to stimulate T cells and mediate cellular immune response. On the other hand, antigen proteins can be recognized and captured by antigen-specific high affinity immunoglobulins on the B cell surface, then provoke humoral immune response, which is assisted by the pre-activated antigen-specific CD4+ T cells (Fig. 11) [118].

Figure 11. General principles of immune responses induced by DNA vaccines [118]. MHC: major histocompatibility complex.

Compared with the traditional vaccines, the immunogenicity of DNA vaccines is quite weak. To enhance the immunogenicity, several strategies are utilized: ① stronger promoters are adopted for the cloning of recombinant plasmid, such as APC-specific promoters; ② optimize the delivery route, transducing DNA vaccines into antigen-presenting cells (APCs), such as dendritic cells, may significantly promote the cytotoxic responses; ③ optimization of multiple antigen sequences; ④ adjuvants are used to prevent tolerance induction and facilitate the innate immune signals induced by DNA vaccines, including alum, liposome, nanoparticles, cytokines, chemokines and pathogen-recognition receptor (PRR) agonists; ⑤ circumvent potential inhibitory effects of the vector [119].

Although DNA vaccines have not been applied widespread due to their weak immunogenicity, promising outcomes are achieved based on improvements the priming high-level antigen specific antibody responses. Some of them are listed in the following Table 7 [120, 121].

Table 7. Examples of clinical trials using DNA vaccine [121]

Disease	Antigen	Delivery route	Status	Outcome	Reference
HIV	EnV, ReV	Intramuscular	Phase I	Ab, CTL, T proliferative, chemokine release was observed	[122]
HIV	gp120, gp160	Intramuscular	Phase I	No Ab response and cellular response were observed	[123]
HIV	ChAdV63	Intramuscular	Phase II	No intervention-related serious adverse events	[124]
Malaria	38 cytotoxic T cell epitopes and 16 helper T cell epitopes derived from P. falciparum antigens	Electroporation	Phase I	poorly immunogenic	[125]
Anal	HPV-16 E7 fragment	Intramuscular	Phase I	10/12 patients developed antigen-specific immune responses	[126]
B-cell lymphoma	Idiotype	Intramuscular	Phase I/II	1/12 patient developed T-cell response to autologous Id following initial immunization course; 6/12 patients developed anti-Id responses following booster immunization	[127]
Breast carcinoma	HER2	Intramuscular	Phase I	3/8 individuals had enhanced CD4+ T cell responses; 3/5 patients have enhanced HER2 Ab responses	[128]
Colorectal cancer	CEA fused to T-helper epitope	intradermal	Phase I	Erythema at injection site increased over time	[129]
Colorectal cancer	CEA (along with HBV surface antigen)	Intramuscular	Phase I	4/17 patients developed Hsp65-specific IL-10 responses	[130]
Melanoma	gp 100 (mouse)	Intramuscular	Phase I	4/27 patients developed gp100 tetramer+CD8+ cells; 5/27 developed IFN γ +CD8+ responses, one of which was tetramer+	[131]

Melanom a	gp 101 (mouse and human)	Intramuscular	Phase I	6/18 patients developed gp100-specific T cell responses	[132]
Melanom a	Tyrosinase (human and mouse)	Intramuscular	Phase I	7/17 patients developed antigen-specific T-cell responses	[133]
Melanom a	MART-1	Intramuscular	Phase I	No enhancement in antigen-specific immune responses	[134]
Melanom a	MART-1 and tyrosinase T-cell epitopes	Intra-lymph node	Phase I	4/19 patients developed immune responses to MART-1	[135]
Melanom a	Epitopes from five melanoma antigens	Intramuscular	Phase I	22/31 patients developed antigen-specific T-cell responses	[136]
Melanom a	gp100	PMED	Phase I	No antigen-specific immune responses detected	[137]
Melanom a	HLA-B7	DNA-containin g liposomes	Phase I	2/22 patients developed enhanced TIL cytotoxicity	[138]
Melanom a	Tyrosinase epitopes	Intra-lymph node	Phase I	11/26 patients had antigen-specific T-cell responses	[115]
NSCLC	L523S	Intramuscular	Phase I	1/10 patient developed antigen-specific antibody response	[139]

4. RNA based vaccines

Though DNA vaccines avoid the anti-vector immunity, long term existence in cells might produce excessive antigens, which might provoke overactive immune responses and exhaust T cells [140]. Thus, another kind of nucleic acid-based vaccines, RNA vaccines, were developed by directly delivering mRNA into cells for the prevention and therapy of diseases. Without the need to entry into cell nucleus, RNA vaccines mainly function in cytoplasm, which might have higher efficiency in delivery [141].

RNA vaccines can be classified into 2 categories: conventional mRNA-based vaccines and self-amplifying mRNA vaccines (SAM, also termed replicons) [142]. Upon entry into cells, conventional RNA vaccines directly generate antigens with nutrients and materials of host cells. On the contrary, SAM vaccines generate antigens by several steps (Fig. 12). SAM can self-replicate in host cells, thus they can mediate high expression levels of antigen. Due to no expression of structural proteins, no virion particle can be produced.

Figure 12. Schematic analysis between conventional mRNA-based vaccines and self-amplifying mRNA vaccines [142]. 1) Conventional mRNA vaccines can be directly translated into antigen. 2) The processes of antigen generation from self-amplifying mRNA vaccines can be divided into four steps: ① The genomic (+)RNA encodes and generates the nonstructural proteins (nsP1, nsP2, nsP3, nsP4), producing a RNA-dependent RNA-polymerase (RDRP) complex; ② RDRP utilizes genomic (+)RNA as template to generate genomic (-)RNA; ③ RDRP utilizes genomic (-)RNA as template to produce genomic (+) RNA and subgenomic RNA; ④ Subgenomic RNA is translated into antigen.

Besides the common immune responses induced by endogenous antigens, mRNA vaccine can elicit a robust type I IFN response, which facilitates CD8⁺ T cell cytolytic capacity and promotes eradication of infected cells [143]. However, some adverse effects are also observed. For example, specific single mutations in nsP1 sequence of alphaviruses, such as A533I, induce elevated Type I IFN expression, but decrease the expression level of antigen and vaccine immunity [144, 145]. To improve the efficacy of RNA vaccines, several strategies are applied: ① Modify or optimize the vaccine backbone, such as 5' cap, poly (A) tail, codon optimization; ② Delivery systems (naked mRNA, formulation with liposomes, lipoplexes, polyplexes, particulate carrier-mediated, electroporation, and gene gun [146]) and route of administration (such as intradermal [147] or intratumor administration [148]); ③ supplementation with small immunomodulatory molecules can also help improve the immune responses provoked by RNA vaccines, such as dexamethasone [149].

As a novel kind of vaccine, RNA vaccines have many advantages: ① Induction of humoral immune responses and cell immune responses; ② Provoke stronger immunity than DNA vaccines; ③ Avoid the anti-vector immunity; ④ Mediate transient expression of antigen; ⑤ No integration into host genome. To date, numerous mRNA vaccines have been translated into clinical trials, and several of them are listed in the following Table 8 [142].

Table 8. Examples of clinical trials using RNA vaccine [142]					
Disease	Antigen	Delivery route	Status	Outcome	Reference
Melanoma	NY-ESO-1, MAGE-A3, tyrosinase and TPTE	Intravenous	Phase I	IFN α and strong antigen-specific T-cell responses were induced	[150]
Non-small cell lung carcinoma (NSCLC)	NY-ESO-1, MAGEC1, MAGEC2, BIRC5, TPBG, and MUC1	Intradermal	Phase I	Improved survival	[151]
Melanoma	Melan-A, Tyrosinase, gp100, Mage-A1, Mage-A3, and Survivin in 21	Intradermal	Phase II	Vaccines are feasible and safe, and induce immune response	[152]
Melanoma	Melan-A, Tyrosinase, gp100, Mage-A1, Mage-A3, and Survivin in 22	Intradermal	Phase I/II	An increase in antitumor humoral immune response was observed in some patient	[153]
Renal cell carcinoma (RCC)	Tumor-associated antigens mucin 1(MUC1), carcinoembryonic (CEA), human epidermal growth factor receptor 2 (Her-2/neu), telomerase, survivin, and melanoma-associated antigen 1 (MAGE-A1)	Intradermal	Phase I/II	Induce CD8+ and CD4+ immune Responses	[154]
Renal cell carcinoma (RCC)	MUC1, CEA, Her2/neu, telomerase, survivin, MAGE-A1	Intradermal	Phase I/II	A clear correlation was observed between survival and immunological responses to TAAs	[155]
Rabies	Rabies virus glycoprotein (CV7201)	Intradermal or intramuscular	Phase I	Boostable functional antibodies against a viral antigen was observed when administered with a needle-free device	[156]
Flu	Hemagglutinin (HA) proteins	Intramuscular	Phase	Protective	[157]

			I	immunogenicity with acceptable tolerability profiles were induced	
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5. Vaccines development for COVID-19/SARS-CoV-2

Coronavirus Disease 2019 (COVID-19) is a novel viral pneumonia caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). First discovered in Wuhan, a city in Hubei province of China, COVID-19 has already broken out throughout the world and posed a great threat to the public health, especially in Europe and North America now. Additionally, person-to-person transmission of COVID-19 disease is reported to be extremely rapid [158-160]. To date, more than two million cases were infected with COVID-19 and over 135,000 deaths occurred. Therefore, it is really urgent and noteworthy to develop the vaccines specific to COVID-19/SARS-CoV-2.

Belonging to the Betacoronavirus genus family, SARS-CoV-2 is 60 ~ 200nm in diameter and encapsidates a large positive-sense, single-stranded RNA virus (26-32kb) with many spikes on the virus capsid (Fig. 13A). The RNA genome of SARS-CoV-2 encodes several accessory proteins and structural proteins, such as nucleocapsid (N) protein, envelope (E) protein, membrane (M) protein, and spike (S) protein (Fig. 13B). Although the detailed mechanism of SARS-CoV-2 infection has not been clearly illuminated, several studies demonstrated that SARS-CoV-2 enters human cells via utilizing spike (S) protein to bind to the angiotensin converting enzyme (ACE2) on the surface of target cell [161, 162].

Figure 13. SARS-CoV-2 capsid structure and genome map. (A) Three-dimensional structure diagram of SARS-CoV-2. (B) Genome organization of SARS-CoV-2 [158]. ORF: open reading frame. E: envelope. M: membrane. N: nucleocapsid. HR1: heptad repeat 1. HR2: heptad repeat 2. SP: signal peptide. NTD: N-terminal domain. RBD: receptor binding domain. S: spike. S1: subunit 1. S2: subunit 2. TM: transmembrane domain.

Since the genome sequences of SARS-CoV were discovered and reported (<https://www.gisaid.org/CoV2020/>), a large number of pharmaceutical enterprises and

research organizations are sparing all efforts to the vaccine development. Different companies utilize different targets and antigen epitopes. Some of the advances are listed in the following Table 9 (from WHO), and most of them focus on viral vector-based vaccines (replicating or non-replicating viral vector-based vaccines), recombinant protein (Spike), and nucleic acid-based vaccines. To date, two COVID-19 vaccines have entered clinical testing to assess the safety and potency of vaccines. One is mRNA-1273, was developed by Moderna Therapeutics and has entered into Phase I clinical trials, encoding a prefusion-stabilized form of Spike (S) protein [163] (<https://www.nature.com/articles/d41587-020-00005-z>). Another vaccine is recombinant AdV vector-based SARS-CoV-2 antigen vaccine, which is developed by Chinese Academy of Military Sciences, Institute of Military Medicine, and has entered into Phase II status. It was predicted that these vaccines can be applied in clinics in a large scale as early as 2021 if they can successfully pass the clinical testing. Although there is a long way for these vaccines to be applied for prevention and therapy of COVID-19, they indeed bring great hope and light to people all over the world.

Table 9 - Advance in COVID-19 vaccine development					
Platform	Type of candidate vaccine	Developer	Coronavirus target	Current stage of clinical evaluation/regulatory status- Coronavirus candidate	Same platform for non-Coronavirus candidates
DNA	DNA plasmid vaccine Electroporation device	Inovio Pharmaceuticals	COVID-19	Pre-Clinical	Lassa, Nipah; HIV; Filovirus; HPV; Cancer indications; Zika; Hepatitis B
DNA	DNA	Takis/Applied DNA Sciences/Envivax	COVID-19	Pre-Clinical	
DNA	DNA plasmid vaccine	Zydus Cadila	COVID-19	Pre-Clinical	
Live Attenuated Virus	Deoptimized live attenuated vaccines	Codagenix/Serum Institute of India	COVID-19	Pre-Clinical	HAV, InfA, ZIKV, FMD, SIV, RSV, DENV
Non-Replicating Viral Vector	MVA encoded VLP	GeoVax/BravoVax	COVID-19	Pre-Clinical	LASV, EBOV, MARV, HIV
Non-Replicating	Ad26 (alone or with MVA)	Janssen Pharmaceutical	COVID	Pre-Clinical	Ebola, HIV, RSV

g Viral Vector	boost)	al Companies	-19		
Non-Replicating Viral Vector	ChAdOx1	University of Oxford	COVID-19	Pre-Clinical	Influenza, TB, Chikungunya, Zika, MenB, plague
Non-Replicating Viral Vector	Adenovirus-based NasoVAX	Altimmune	COVID-19	Pre-Clinical	Influenza
Non-Replicating Viral Vector	Ad5 S (GREVAX™ platform)	Greffex	COVID-19	Pre-Clinical	MERS
Non-Replicating Viral Vector	Oral Vaccine platform	Vaxart	COVID-19	Pre-Clinical	InfA, CHIKV, LASV, NORV; EBOV, RVF, HBV, VEE
Non-Replicating Viral Vector	Viral-vectored based	CanSino Biologics	COVID-19	Pre-Clinical	
Protein Subunit	Drosophila S2 insect cell expression system VLPs	ExpreS2ion	COVID-19	Pre-Clinical	
Protein Subunit	S protein	WRAIR/USAMRIID	COVID-19	Pre-Clinical	
Protein Subunit	S-Trimer	Clover Biopharmaceuticals Inc./GSK	COVID-19	Pre-Clinical	HIV, REV Influenza
Protein Subunit	Peptide	Vaxil Bio	COVID-19	Pre-Clinical	
Protein Subunit	Li-Key peptide	Generex/EpiVax	COVID-19	Pre-Clinical	Influenza, HIV, SARS-CoV
Protein Subunit	S protein	EpiVax/Univ. of Georgia	COVID-19	Pre-Clinical	H7N9
Protein Subunit	S protein (baculovirus	Sanofi Pasteur	COVID	Pre-Clinical	Influenza, SARS-CoV

	production)		-19		
Protein Subunit	Full length S trimers/ nanoparticle + Matrix M	Novavax	CO VID -19	Pre-Clinical	RSV; CCHF, HPV, VZV, EBOV
Protein Subunit	S protein clamp	University of Queensland/GSK	CO VID -19	Pre-Clinical	Nipah, influenza, Ebola, Lassa
Protein Subunit	S1 or RBD protein	Baylor, New York Blood Center, Fudan University	CO VID -19	Pre-Clinical	SARS, MERS
Protein Subunit	Subunit protein, plant produced	iBio/CC-Pharming	CO VID -19	Pre-Clinical	
Replicating Viral Vector	Measles Vector	Zyklus Cadila	CO VID -19	Pre-Clinical	
Replicating Viral Vector	Measles Vector	Institute Pasteur	CO VID -19	Pre-Clinical	West Nile, Chik, Ebola, Lassa, Zika
Replicating Viral Vector	Horsepox vector	Tonix Pharma/Southern Research	CO VID -19	Pre-Clinical	Smallpox, monkeypox
RNA	mRNA	China CDC/Tongji University/Starmina	CO VID -19	Pre-Clinical	
RNA	mRNA	Moderna/NIAID	CO VID -19	Phase I clinical trial	Multiple candidates
RNA	mRNA	Arcturus/Duke-NUS	CO VID -19	Pre-Clinical	Multiple candidates
RNA	saRNA	Imperial College London	CO VID -19	Pre-Clinical	EBOV; LASV, MARV, Inf (H7N9), RABV
RNA	mRNA	Curevac	CO VID -19	Pre-Clinical	RABV, LASV, YFV; MERS, InfA, ZIKV, DengV, NIPV

Unkno wn	Unknown	University of Pittsburgh	CO VID -19	Pre-Clinical	
Unkno wn	Unknown	University of Saskatchewan	CO VID -19	Pre-Clinical	
Unkno wn	Unknown	ImmunoPreci se	CO VID -19	Pre-Clinical	
Unkno wn	Unknown	MIGAL Galilee Research Institute	CO VID -19	Pre-Clinical	
Unkno wn	Unknown	Doherty Institute	CO VID -19	Pre-Clinical	

6. Tumor/Cancer vaccines

Despite several decades' continuous efforts on tumor therapy with radiation treatment and chemotherapy, serious adverse effects, including nausea, vomiting, physical weakness, mental malaise, sweating, decreased white blood cells and platelets, are really painful and intolerable. Tumor vaccines are vaccines using tumor-specific antigens (TSAs) to provoke the host immune system to specifically eliminate and suppress tumors or cancers, exhibiting promising advantages over traditional radiation and chemotherapy. Several kinds of tumor vaccines are developed for the therapy of tumor as shown in Fig. 18 [164], including nucleic acid vaccines (DNA vaccines and RNA vaccines), protein/peptide vaccines, and cell-based vaccines with tumor antigens.

Figure 14. Schematic representation of tumor (cancer) vaccines [164].

Nucleic acid-based vaccines can be taken up by APCs and translated into specific antigen to provoke host immune system, such as Tyrosinase Related Protein 1 (TYRP1/gp75) vaccine for the treatment of melanoma [165]. Protein/peptide-based vaccines are tumor specific antigens protein or epitope, which can directly stimulate immune system, such as HSPPC-96 vaccine (Oncophage) for the treatment of melanoma, gastric cancer, renal cell cancer, lymphoma, and pancreatic cancer [166]. Cell-based vaccines are autologous dendritic cells or other immune cells with insertion of tumor antigen genes or transfected with tumor antigens or peptides, such as dendritic cell vaccine, provenge (sipuleucel-T), targeting PAP for the therapy of prostate cancer [167]. A large number of tumor vaccines have been translated into clinics and encouraging therapy outcomes have been achieved (Table 10).

Table 10. Examples of vaccines against tumor						
Disease	Antigen/target	Vaccine classification	Delivery route	Status	Outcome	References
Prostate cancer	PAP	DNA vaccine	Intravenous	Phase III	Improved survival	[167]
Prostate cancer	VEGF	DNA vaccine	Oral	Phase	Vaccine were	[168]

	Receptor 2			e I	well-tolerated and T effector was increased	
NSCLC	MUC1	Protein/peptide-based vaccines	Intravenous	Phase III	Tecemotide might have some adverse events on patients who initially receive concurrent chemoradiotherapy	[169]
NSCLC	MUC1	Protein/peptide-based vaccines	Subcutaneous	Phase III	Safe, but adverse events existed	[170]
Melanoma	HSPPC-96	Protein/peptide-based vaccines	Intradermal	Phase I/II	Feasible and safe. Modest immune response and anti-tumor activity were observed	[171]
Ovarian carcinoma, glioblastoma, pancreatic carcinoma, stomach carcinoma	Multiple tumor-associated antigens (TAAs)	Tumor cell vaccine	Intradermal	Phase I/II	Antitumor immune memory and patient survival were improved	[172]
Prostate cancer	PAP	Recombinant adenoviral vector-based vaccine	Subcutaneous	Phase I/II	Safe with no serious vaccine-related adverse events, and anti-PSA T-cell responses were induced	[172]

Melanoma	IL-2	Autologous tumor cell vaccine via Ad vector-mediated IL-2 transfection	Intradermal/subcutaneous	Phase I	Safe and tolerate	[173]
Melanoma	MART-1 or gp100	Recombinant adenoviral vector-based vaccine	Intramuscular/subcutaneous	Phase I	Safe, but presenting high levels of neutralizing antibody.	[174]
Melanoma	GM-CSF	Irradiated autologous tumor cell vaccine via Ad vector-mediated GM-CSF transfection	Intradermal/subcutaneous	Phase I	Well tolerated and induce anti-tumor immune response	[175]
Melanoma	MART-1	Autologous dendritic cell vaccine via Ad vector-mediated MART-1 transfection	Intradermal	Phase I/II	Safe and immunogenic	[176]
Solid tumor	HER2	DNA vaccine and adenoviral vector-based vaccine	Intramuscular	Phase I	Well tolerated and without any serious adverse events	[79]
Non-muscle-invasive bladder cancer (NMIBC)	IFN α /Syn3	Recombinant adenoviral vector-based vaccine	Intravesical	Phase II	Well tolerated	[177]
Non-muscle-invasive bladder cancer (NMIBC)	IFN α 2b/Syn4	Recombinant adenoviral vector-based vaccine	Intravesical	Phase Ib	Promising drug efficacy was shown	[178]
Non-muscle-inv	IFN α /Syn3	Recombinant	Intravesical	Phas	Well tolerated	[179]

asive bladder cancer (NMIBC)		adenoviral vector-based vaccine		e I	with no dose limiting toxicity	
Colorectal cancer	CEA	Recombinant adenoviral vector-based vaccine	Subcutaneous	Phase I/II	Safe and immunogenic	[179]
Colorectal cancer	CEA	Recombinant adenoviral vector-based vaccine	Subcutaneous	Phase I	Minimal toxicity	[180]
Hepatocellular cancer	AFP	DNA vaccine and adenoviral vector-based vaccine	Intramuscular	Phase I	Safe and immunogenic	[181]
B-cell lymphosarcoma	Telomerase	Recombinant adenoviral vector-based vaccine	In femoral biceps	Phase I	Safe and prolong the survival time	[182]

As is known to all, there are many somatic mutations in tumors varying in different individuals, and more and more highly heterogeneous neoantigens are discovered and identified through next generation sequencing (NGS) technologies. On the basis of tumor mutation profiles, personalized cancer vaccines are designed and developed to activate immune system against cancer by targeting specific epitopes of neoantigens (Fig. 19). Once delivered into the body with adjuvant, personal vaccines provokes host immune responses through the following processes: ① Neoantigen-specific peptides inside personal vaccines are captured and processed by APCs; ② Activated APCs migrate to lymph nodes and present neoantigens to T cells with MHC molecules; ③ Neoantigens are recognized and bound by T cell receptor, thus priming and activating cell immunity; ④ Neoantigen-specific T cells are expanded, migrate and infiltrate to tumor microenvironment; ⑤ Neoantigen-specific T cells kill tumor cells with neoantigens, leading to the release of more antigens, which elicits adaptive immune memory and augments the immune responses. So far, personal vaccines have achieved encouraging anti-tumor effects in the pre-clinical studies with mouse models and clinical trials. For example, using whole-exome and transcriptome sequencing and mass spectrometry analysis, more than 1300 amino acid changes were identified in MC-38 and TRAMP-C1 murine tumor models, and vaccination with mutated peptides exhibits remarkable and sustainable inhibition of tumor growth

[183]. For human melanoma therapy, a neoantigen was discovered via whole exome sequencing and HLA binding prediction algorithm, and dendritic cell vaccine with the neoantigen significantly enhances the T cell immune response to the neoantigen in some patients [184].

Figure 15. Schematic of strategies and principles of personal neoantigen vaccines [164]. (I) Get the tumor specimen from patient and extract the DNA. (II) Characterize the non-synonymous mutations via NGS. (III) Prepare the personal neoantigen vaccine in vitro and deliver the vaccine to patients with adjuvant.

7. Summary

In summary, different kinds of vaccines have their specific advantages and disadvantages (Table 12), and people should select the most suitable vaccines according to their demands. Although nucleic acid-based vaccines need short-term and little cost, DNA-based vaccines provoke mild immune responses, and no available mRNA vaccines have been applied into clinics, which really restrict the application of nucleic acid-based vaccines. Viral vector-based vaccines, especially adenoviral vector-based vaccines, stimulate relatively stronger immune responses, showing unique superiority in vaccine development. However, the COVID-19 is raging across the world now, which makes the vaccines for COVID-19 urgently demanded. At this fierce moment, time is really precious for life, which might give a chance for mRNA vaccine. At the same time, as a kind of effective and efficacious vaccines, viral vector-based vaccines are also promising and with great prospect for COVID-19 vaccine development.

Table 12 - Comparison among several kinds of novel vaccines			
Vaccine classification	Viral vector-based vaccines	DNA vaccine	RNA vaccine
Immune responses	Induce humoral and cellular immunity	Induce humoral and cellular immunity	Induce humoral and cellular immunity
Immunogenicity	Varies with different vectors	Weak	Weak

Advantages	① highly efficient in gene transduction; ② mediate specific gene delivery to target cells; ③ induce of both humoral and cell-mediated immune responses; ④ better efficacy and safety; ⑤ just need low administration dose; ⑥ easy to be applied into large-scale manufacturing; ⑦ possessing widespread potential target diseases, ranging from infectious diseases to cancers	① Induction of humoral immune responses and cell immune responses; ② Avoid the anti-vector immunity; ③ Easy to produce in a large scale	① Induction of humoral immune responses and cell immune responses; ② Provoke stronger immunity than DNA vaccines; ③ Avoid the anti-vector immunity; ④ Mediate transient expression of antigen; ⑤ No integration into host genome; ⑥ Easy to produce in a large scale
Drawbacks	Pre-existing immunity and neutralizing antibodies (AAV and AdV); tumorigenicity (LV)	Induce weak immunity	Induce weak immunity
Suitable time	AAV and LV: long-term stable expression; AdV: transient	Transient	Transient
Delivery system	Viral vector	Naked nucleic acids, formulation with liposomes, lipoplexes, polyplexes, particulate carrier-mediated, electroporation, and gene gun	Naked nucleic acids, formulation with liposomes, lipoplexes, polyplexes, particulate carrier-mediated, electroporation, and gene gun

Optimization	AAV: rational design of capsid; AdV: modify fiber protein to alter tissue tropism; LV: develop integration-free vector	① Utilizing stronger promoters; ② Optimize the delivery route; ③ Optimization of multiple antigen sequences; ④ Adjuvants are used to prevent tolerance induction and facilitate the innate immune signals; ⑤ circumvent potential inhibitory effects of the vector	① Modify or optimize the vaccine backbone; ② Optimize the delivery systems and route of administration; ③ supplementation with small immunomodulatory molecules to improve the immune responses
Clinical usage	Yes	Not yet	Not yet

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