A new generation of vaccines based on alphavirus self-amplifying RNA

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DNA or mRNA vaccines have potential advantages over conventional vaccines since they are easier to manufacture and have higher safety profiles. In particular, self-amplifying RNA (saRNA) derived from alphavirus expression vectors has shown to be very efficient to induce humoral and cellular responses against many antigens in preclinical models, being superior to non-replicating mRNA and DNA. This is mainly due to the fact that saRNA can provide very high expression levels and simultaneously induces strong innate responses, potentiating immunity. saRNA can be administered as viral particles or DNA, but direct delivery as RNA represents a safer and more simple approach. Although saRNA can be delivered as naked RNA, *in vivo* transfection can be enhanced by electroporation or by complexing it with cationic lipids or polymers. Alphavirus saRNA could have broad application to vaccinate against human pathogens, including emerging ones like SARS-CoV-2, for which clinical trials have been recently initiated.

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Introduction

The use of nucleic acids to induce immune responses against encoded antigens represents a very attractive type of vaccines, compared to more classical approaches based on peptides, proteins, attenuated and inactivated viruses, or viral vectors expressing antigens. The first nucleic acid vaccines to be tested in animals were based on plasmid DNA, resulting in induction of both humoral and cellular immune responses against a great variety of antigens [1]. Soon it became clear that to obtain optimal results, DNA had to be delivered *in vivo* by complexing it with different molecules, like cationic lipids, polymers or peptides, that could compact it and facilitate entry through the cytoplasmic membrane [1]. Physical methods, like electroporation or gene gun, were also effective at enhancing DNA delivery. However, a DNA vaccine presents some drawbacks, for example, it needs to reach the cell nucleus to be functional, a process that is usually not very efficient. At the same time, its presence in the nucleus gives rise to the possibility of genomic integration and induction of oncogenic processes. Besides good results in preclinical models, DNA vaccination has been relatively modest in inducing immune responses in humans, and no DNA vaccine has been approved for clinical use so far [2]. An alternative to DNA is the use of messenger RNA (mRNA). This molecule presents the same problems for delivery as DNA, but it does not need to reach the nucleus to be expressed, facilitating transfection. In addition, mRNA cannot integrate, increasing the safety profile of this approach. However, mRNA can be easily degraded by extracellular ribonucleases present in skin and blood. This problem can be avoided by complexing mRNA with compounds able to protect it against degradation and facilitate cellular uptake, as will be discussed in this review. Besides these advantages, mRNA vaccination has not demonstrated efficacy until recently, thanks to the development of new technologies to synthesize more stable RNA molecules and new methodologies for efficient *in vivo* delivery [3]. A type of mRNA that has shown extraordinary properties to induce immune responses is the so-called self-amplifying RNA (saRNA). saRNA is derived from the genome of certain viruses like alphaviruses and flaviviruses and has the capacity of self-amplification due to the fact that it expresses a viral replicase (Rep), while the genes coding for the viral structural proteins have been substituted by the transgene of interest [4]. Most saRNAs used in vaccination studies derive from alphaviruses, including Venezuelan equine encephalitis virus (VEEV), Semliki Forest virus (SFV), and Sindbis virus. As depicted in Figure 1, when saRNA enters the cell cytoplasm, it will translate Rep, which will copy this long mRNA into a complementary negative strand RNA that will be used by Rep to make more saRNA. Simultaneously, Rep recognizes a subgenomic promoter in the negative strand from which it will

Figure 1
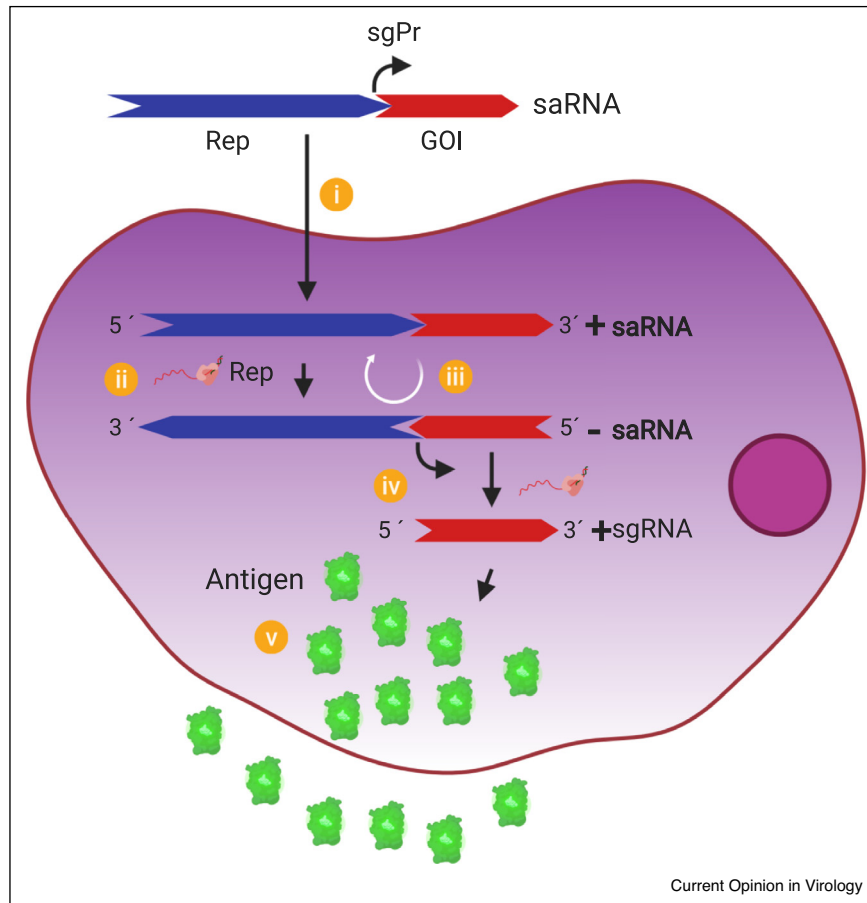
saRNA vectors based on alphavirus. The saRNA vector is a positive strand RNA containing the genes coding for the viral replicase (Rep) and the gene of interest (GOI) downstream of a subgenomic promoter (sgPr). Upon entry of saRNA into cells (i) Rep can be translated, being able to use saRNA as template to make a complementary negative saRNA (-saRNA) strand (ii). Rep can also use this negative RNA as template to make more saRNA (+saRNA), allowing its self-amplification (iii). In addition, Rep can recognize the sgPr in the negative strand from which a subgenomic mRNA (+sgRNA) of positive polarity is synthesized (iv). sgRNA can be translated to produce the desired antigen at very high levels, which will be secreted if having an appropriate signal peptide (v). Both +saRNA and +sgRNA contain a cap at the 5ʹend and are polyadenilated (not shown).

make a smaller mRNA (subgenomic RNA). This mRNA will be produced at levels ten-fold higher than those of genomic RNA, leading to high production of antigen *in vivo*. This, together with the intrinsic properties of saRNA in inducing innate immune signals, like activation of several Toll-like receptors (TLRs) within cells, contributes to the generation of very strong immune responses. In addition, duration of expression from saRNA delivered encapsulated in lipid nanoparticles is longer than the one obtained with mRNA, being able to last almost two months *in vivo* when expressing a reporter gene [5]. Although saRNA can also be delivered by packaging it into viral particles (VPs) or by launching its expression in cells from a plasmid [6], this review will focus on direct delivery of saRNA for vaccination.

Vaccines based on naked saRNA

Naked saRNA is the simplest strategy to deliver RNA into cells. The first evidence that naked saRNA could be used for vaccination was reported in 1994 by Zhou et al. [7], when they showed that intramuscular (IM) injection of 50 μg of SFV RNA carrying influenza virus nucleoprotein (SFV-NP) induced specific humoral responses in mice, although antibody titers were lower than those obtained with 10^6 SFV-NP VPs. A few years later, Ying *et al.* [8] demonstrated that with only 0.1 μg of SFV expressing β-galactosidase (β-gal) RNA given IM it was possible to elicit antibody and CD8 T cell responses. This type of immunization was able to protect mice from a challenge with colon tumor cells expressing β-gal, used as a surrogate tumor antigen. Other groups have shown that naked saRNA can induce immune responses in mice against human pathogens like rabies virus [9], influenza

Table 1

Relevant recent saRNA-based vaccination strategies

Vector[a]	Delivery[b]	Route[c]	Cargo gene[d]	Species	Results	Ref.
N.I.	Nak. RNA/PEI	IM	HA	Mouse	Protection from influenza virus challenge[e]	[10••]
SFV			HIV mosaic ag.		Plurifunctional CD4+ and CD8+ T cells	[12]
ta-SFV	Nak. RNA	ID	HA		Protection from influenza virus challenge	[41••]
VEEV	EP & LNP		Luc		Expression: EP, days 3−10; LNP, peak 24h	[16]
	EP			Pig	High expression for 12 days	[17•]
				Mouse	Optimal expression in tail base versus flank	[19•]
	EP+RNase inh.				RNase inh. increased reproducibility	[19•]
	LNP	IM	HIV ag.-lumaz.		High HIV gp120-antibodies	[37]
			SARS-2 spike		SARS-2 neutralizing antibodies/cell response	[45••]
	LION			Mouse/NHP	SARS-2 neutralizing antibodies/cell response	[46••]
	LNPout		HIV gp140	Mouse	Equivalent antibody versus LNP with RNA inside	[25]
		Exp	Luc/GFP	Hum skin exp	Cephalin LNPs increased expression sevenfold	[35]
	Cationic NLP	IM	Luc	Mouse	Enhanced complexation & delivery	[34]
	NLC		SEAP/ZIKV ag.	Mice/G. pig	Protection against lethal ZIKV challenge	[33••]
	Neutral LPP		GFP/Luc/HA	Mouse	Specific and functional T cell responses	[32•]
	CAFs/PEI		Chlamydia ag.		Immune responses, unaffected by TLR-agonists	[36]
	pABOL		HA		Protection from influenza virus challenge	[40]
	CNE		VEEV TC-83		Full protection against VEEV challenge	[30••]
cVEEV			Viral ag.	Rodents/NHP	Specific T cell and antibody responses	[24]
			HA	Mouse/Ferret	Protection against heterologous virus challenge	[26]
			NP+GM-CSF	Mouse	Enhanced APC recruitment & virus protection	[27•]
	Mannose-LNP	IM/ID	HA		Enhanced APC uptake & immune responses	[31]
VEEVm	LNP	IT	IL-2		Enhanced expression & antitumor effects	[44]

[a] N.I., not indicated; ta, *trans*-amplifying RNA; SFV, Semliki Forest virus; VEEV, Venezuelan equine encephalitis virus; cVEEV chimeric VEEV-Sindbis RNA vector; VEEVm, mutant VEEV.

[b] Nak, naked; EP, electroporation; inh, inhibitor; LNP, lipid nanoparticle; LION, Lipid InOrganic Nanoparticles; LNPout, LNP having RNA outside; NLP, cationic nanolipoprotein particles; NLC, nanostructured lipid carrier; LPP, lipopolyplexes; CAF, cationic adjuvant formulations; PEI, polyethylenimine; CNE, cationic nanoemulsion.

[c] IM, intramuscular; ID, intradermal; Exp, skin explant; IT, intratumoral.

[d] HA, influenza virus hemagglutinin; ag., antigen; Luc, luciferase; lumaz. lumazine; SARS-2, SARS-CoV-2; SEAP, secreted human embryonic alkaline phosphatase; ZIKV, Zika virus; VEEV TC-83, attenuated VEEV strain; Viral ag, antigens from several viruses; NP, influenza virus nucleoprotein; IL-2, interleukin-2.

[e] Hum skin exp, human skin explant; G. pig, guinea pig; NHP, non-human primates.

virus [10••] or human immunodeficiency virus (HIV) [11,12]. In particular, Moyo *et al.* cloned highly conserved regions of HIV-1 gag and pol proteins into a SFV vector to generate mosaic saRNA vaccines, which were administered IM, generating specific T cell responses [12]. Interestingly, these responses had different time-courses compared to virus-based immunization, mediating a gradual induction of T cells during five weeks, with sustained persistence. It was suggested that this delay in the immune response could be due to a lack of immunogens in the first stage of treatment, until saRNA could be translated. Despite these encouraging results, several groups have shown that the potency of naked saRNA vaccines can be greatly improved by electroporation [13], or by complexing them with lipid or polymer formulations [10••,12], as it will be discussed (Table 1 and Figure 2).
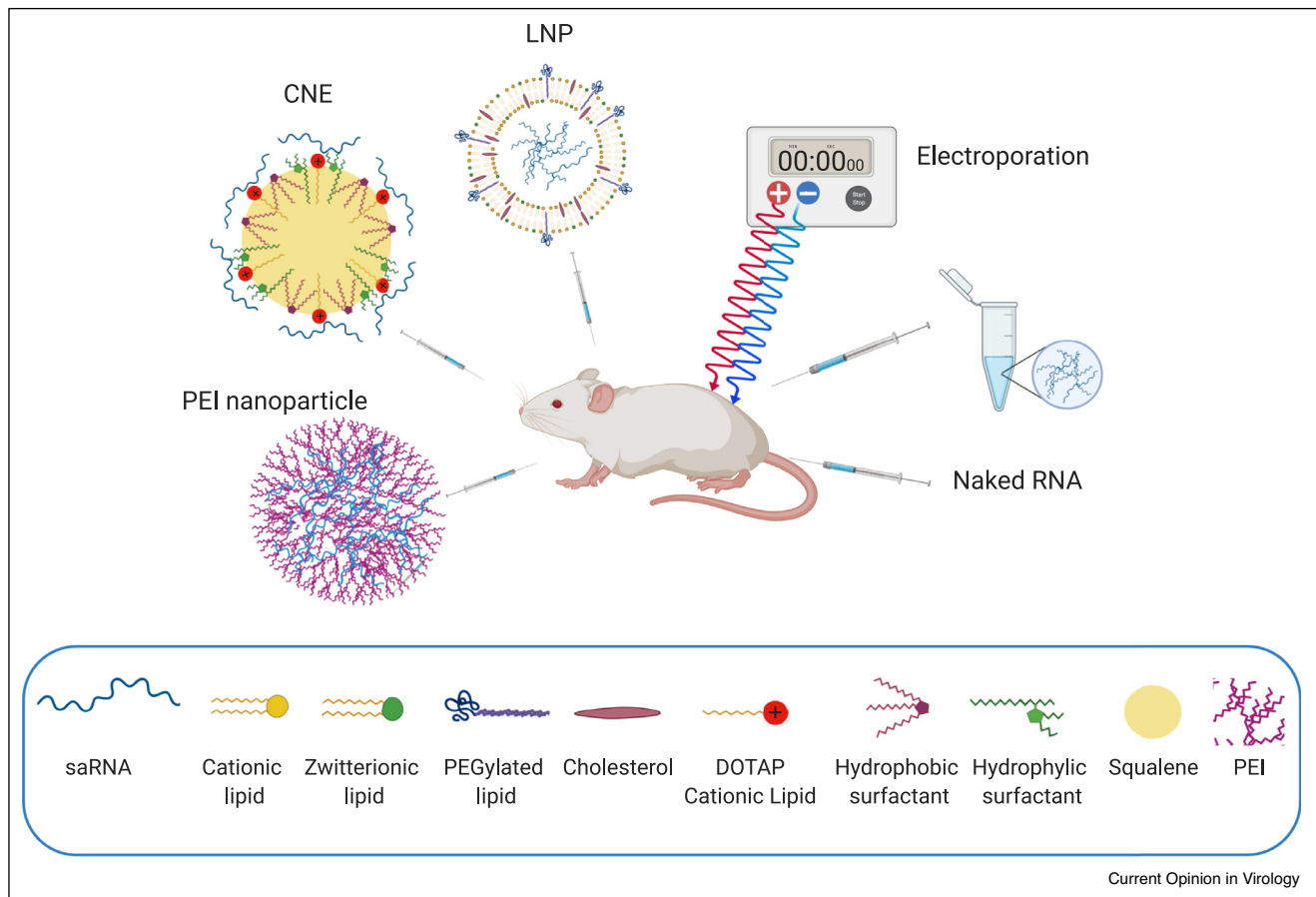
Vaccines based on saRNA electroporation

In vivo electroporation (EP) can enhance the potency of saRNA vaccines, by increasing transfection efficiency as shown for the first time by Piggott *et al.* [14]. In this sense, the skin is a very attractive tissue for immunization, as it is highly immunocompetent and easily accessible, making intradermal (ID) EP a non-invasive procedure. The

enhancement of immune responses by saRNA EP was confirmed by Johansson *et al.* [13] using SFV vectors expressing β-gal or luciferase (luc) fused to a CD8 T cell ovalbumin epitope, respectively. This study showed that conventional mRNA-immunized mice failed to develop any detectable immune responses, even in combination with EP, indicating that RNA replication plays a major role in the induction of immunity. The ability of EP to induce immune responses against human pathogens, like HIV, has been shown using a chimeric VEEV saRNA engineered to contain the 3′terminal untranslated sequences of Sindbis virus (cVEEV), which was injected IM in mice [15]. Again, the authors showed that EP mediated higher protein expression and enhanced antigen-specific cellular and humoral immune responses than injection of naked saRNA without EP.

To get a deeper insight into the mechanism by which EP enhances saRNA immune responses, Huysmans *et al.* [16] have recently characterized the expression kinetics and innate immune responses induced by ID delivery of a VEEV-luc saRNA, using as control conventional mRNA and a plasmid expressing the same protein (pDNA). Expression from both saRNA and pDNA was increased

Figure 2

Methodologies for *in vivo* delivery of saRNA. These include (from left to right) direct injection of naked saRNA formulated in buffer, electroporation, or the use of complexes based on cationic lipids or polymers. In this last case a representative lipid nanoparticle (LNP) containing saRNA inside, a cationic nanoemulsion (CNE) having saRNA outside and a nanoparticle in which saRNA is complexed with polyethylenimine (PEI nanoparticle) are represented. The most common components present in these particles are indicated below.

with EP, although this effect was significantly more pronounced for saRNA. In this study, they also observed striking differences in the expression time-course between EP and lipid nanoparticles (LNPs) used for saRNA delivery. While saRNA EP resulted in a plateau expression between days 3–10, expression after LNP delivery peaked at 24 hour followed by a sharp drop. This could be explained by the fact that saRNA delivered by EP generated lower innate immune responses compared to LNP delivery.

Most preclinical studies using mRNA have been performed in mice, which may not mimic humans. In the case of pDNA-based vaccines, it is well-known that their efficacy is much lower in larger animals and humans than in mice [2]. A first attempt has been made in pigs using saRNA delivered ID in combination with EP [17•]. In this study, the expression obtained with a VEEV-luc saRNA was longer compared to pDNA and non-replicating mRNAs. While saRNA maintained expression during at least 12 days, pDNA showed a maximum of expression at day one followed by a steep drop at day two, possibly due to epigenetic silencing [18]. The fact that porcine and human skin have similarities, make these results more translatable to humans.

Although the skin is a promising tissue for immunization, it has some shortcomings that should be considered. On one hand, it is a very large and heterologous organ, hence caution should be taken when choosing the immunization site. In mice, EP of a VEEV-luc saRNA at the tail base resulted in a significantly higher and longer luc expression compared with the same administration at the flank [19•]. This observation emphasizes the fact that location of the ID immunization should be chosen with thoughtfulness in human clinical trials. On the other hand, the skin contains high levels of RNases that act as a natural protective mechanism against pathogens, which might

be a drawback for RNA-based vaccines. Preclinical studies delivering saRNA ID in combination with EP had shown a high variability in expression levels, which could be due to RNA degradation. The addition of placental RNase inhibitor to saRNA before ID injection was shown to increase efficacy and reproducibility of expression in mice [19•].

Vaccines based on saRNA conjugated to LNPs

LNPs have demonstrated to be a powerful tool for saRNA delivery, generating several vaccine platforms against infectious diseases. This success has been possible thanks to critical advances in LNP formulations focused at improving i) stability ii) infectivity, iii) cytosolic delivery, iv) low immunogenicity, v) capacity to induce humoral and cellular immune responses, and vi) low reactogenicity.

The first report on the use of LNPs to deliver saRNA was described by Geall et al. [5] using a technology previously developed for siRNA delivery, based on the use of the ionizable cationic lipid 1,2-dilinoleyloxy-3-dimethylaminopropane (DLinDMA) as a main LNP component. This type of LNP-delivered saRNA vaccines, named SAM (for self-amplifying mRNA) platform, have shown great potential to generate immune responses against influenza virus [20–22] and *Toxoplasma gondii* [23]. The group of Dr. Geall (Novartis Vaccines, Cambridge, MA) has also described an alternative LNP system based on a cationic nanoemulsion (CNE), able to bind saRNA, enhancing its delivery and increasing the potency of the vaccine [24]. CNE is composed of cationic lipid DOTAP (1,2-dioleoyl-sn-glycero-3-phosphocholine) emulsified with MF59, a Novartis's proprietary adjuvant based on squalene with a good clinical safety profile. An advantage of CNE formulation is that it can be stockpiled separated from saRNA, which can be admixed before administration. Despite the fact that RNA is exposed on the outside of these particles, it is protected from degradation by RNAses [24]. This protection has also been observed for LNPs based on cationic lipids formulated with saRNA adsorbed to their surface [25].

The CNE platform has shown to be very efficient at inducing immune responses against human pathogens like respiratory syncytial virus [24], human cytomegalovirus [24], influenza virus [26,27•], HIV [24,28], and *Streptococci* [29], using different animal models. In particular, evidence has been provided that HIV vaccination with a relatively low dose of saRNA (50 µg) was both safe and immunogenic in nonhuman primates [28]. The CNE system has even been employed to deliver a live-attenuated VEEV vaccine using the full-length RNA genome of VEEV TC-83 attenuated strain [30••]. This vaccine induced immune responses similar to those of TC-83 VPs, providing 80% protection against VEEV challenge in mice. The advantage of this strategy is that it can

eliminate the need for live-attenuated vaccine production, although it does not prevent the possibility of reversion. To make the vaccine safer, the authors generated a second version in which they completely deleted the TC-83 capsid gene. Despite being less immunogenic, this second formulation resulted in significant protection against VEEV challenge [30••].

Regarding the mechanism by which saRNA vaccination can induce potent immune responses, it has been proposed that upon IM vaccination antigen is expressed in muscle cells and then transferred to antigen presenting cells (APCs), suggesting a cross-priming mechanism able to prime CD8 T-cells [21]. Following this rationale, Manara et al. investigated the possibility to enhance saRNA-induced immune responses by increasing recruitment of APCs at the injection site [27•]. For that purpose, they combined a saRNA expressing influenza virus nucleoprotein (NP) with a second saRNA expressing granulocyte-macrophage colony-stimulating factor (GM-CSF), a chemoattractant for APCs. Vaccination of mice with this saRNA combination formulated in CNE significantly improved NP-specific cellular responses and provided increased protection against influenza virus challenge. A different strategy to increase APCs saRNA uptake was described by Goswami et al. [31], based on the inclusion of mannose-cholesterol amine conjugates in LNPs. Since APCs express significant amounts of mannose receptors on their surface, this strategy increased humoral and cellular responses against influenza hemagglutinin (HA) expressed from a saRNA. A similar approach has been used by Perche et al. [32•] using neutral lipopolyplexes (LPPs), a tripartite formulation with saRNA, a cationic polymer, and anionic liposomes, including a mannosylated lipid to enhance transfection of dendritic cells (DCs).

A great effort has been performed in optimizing LNP formulations in order to obtain more stable particles and higher *in vivo* expression. Of note, Erasmus et al. [33••] generated a highly stable nanostructured lipid carrier (NLC) based on a mixture of a solid lipid (glyceryl trimyristate-dynasan 114) and liquid oil (squalene) able to form a semi-crystalline core upon emulsification. As in the case of CNE, this NLC could be stored separated from saRNA and admixed at the time of use, greatly facilitating its production. In addition, they showed that combining it with only 10 ng of a VEEV saRNA expressing Zika virus antigens could completely protect mice against a lethal viral challenge. Using a different approach to enhance complexation and delivery of saRNAs, He et al. [34] developed cationic nanolipoprotein particles, based on a discoidal lipid bilayer stabilized by high-density lipoprotein. Interestingly, these complexes required less amount of cationic lipids compared to other LNP platforms and were efficient for *in vivo* delivery of saRNA.

Regarding strategies to increase *in vivo* expression, Blakney *et al.* [35] observed that by including cephalin (a zwitterionic lipid) in LNP formulations containing VEEV saRNA, luc expression in human skin explants was increased by sevenfold. Interestingly, most transfected cells were immune cells, which highlights the potential of this approach for ID vaccination. The same group tried to optimize immune responses by using cationic adjuvant formulations combined with TLR 7/8 agonists, using saRNA expressing the major outer membrane protein (MOMP) of *Chlamydia trachomatis* [36]. Despite obtaining good MOMP-specific cellular and humoral responses, immunogenicity was unaffected by TLR-agonists incorporation, and self-adjuvanting effects of saRNA seemed to dominate the immune response. An interesting approach to generate broadly neutralizing antibodies against HIV was based on the use of LNPs to deliver a VEEV saRNA expressing a gp120 domain fused to lumazine synthase, a bacterial protein which self-assembles into 60-mer protein nanoparticles [37]. This strategy elicited high titers of gp120-specific antibodies in mice, and increased levels of antigen-specific germinal center B cells compared to protein immunization, representing a vaccine with potential interest for HIV.

Vaccines based on saRNA conjugated to polyethylenimine (PEI)

Besides LNPs, mRNAs can also be efficiently delivered if compacted into small particles using cationic polymers like PEI, which contain primary amines that facilitate RNA condensation, protect RNA and enhance cellular uptake. Démoulins *et al.* [38] showed for the first time that saRNA could be complexed with linear PEI to be efficiently delivered to DCs, inducing both humoral and cellular immune responses *in vivo* against influenza virus HA and NP proteins. In a follow-up publication, they fine-tuned PEI complexes in order to improve saRNA delivery [39]. Thus, they found that three parameters were of paramount importance: (i) PEI molecular weight; (ii) saRNA:PEI (weight:weight) ratio; and (iii) inclusion of cell penetrating peptides. Using optimized PEI complexes co-administered with a STING-agonist (c-di-AMP) they were able to induce immune responses in pigs against influenza virus proteins expressed from a saRNA. Although in these two studies the authors used saRNA derived from the pestivirus classical swine fever virus, these strategies could be equally valid to deliver alphavirus-based saRNA. Indeed, a medium-length PEI-based formulation has been used to efficiently deliver an alphavirus saRNA expressing influenza virus HA, being able to induce protection in mice against H1N1 influenza challenge using a single dose of 1.5 μg of RNA [10••]. PEI was also used to complex an SFV-based saRNA vaccine expressing HIV antigens, resulting in specific plurifunctional CD4 and CD8 T cell responses that were higher than those obtained with naked saRNA when injected IM in mice. Furthermore, these responses were sustained during 22 weeks following a single vaccine administration [12]. A new type of polymer for saRNA delivery, called pABOL, has been recently developed by the group of Robin Shattock and Molly Stevens at Imperial College London (London, UK) [40]. pABOL is based on a bioreducible, linear, cationic poly(CBA-co-4-amino-1-butanol) polymer that enhances protein expression and cellular uptake *in vivo* compared to commercially available PEI. Interestingly, increasing the molecular weight of pABOL enhances delivery efficiency for saRNA.

## New developments on alphavirus RNA vectors

Besides technological improvements to deliver saRNA, reviewed in the previous sections, some recent studies have addressed the possibility to optimize the vector itself. In particular, Beissert *et al.* [41••] have developed a *trans*-amplifying RNA (ta-RNA) vaccine based on a bipartite SFV system in which the gene of interest is expressed from a saRNA devoid of replicase, providing this one in *trans* by a non-replicating mRNA (mRNA-Rep) (Figure 3). Despite the fact that the ta-RNA system was able to induce good immune responses *in vivo* against influenza virus HA, it did not outperform vaccination with a single saRNA molecule expressing the same antigen. However, this novel ta-RNA system might have advantages for vaccination over the single vector system in terms of versatility and ease of manufacturing, since mRNA-Rep could be produced and stored in advance.

One limitation on the efficacy of saRNA-based vaccines is the fact that they induce strong innate host immune responses, which could limit the intensity and duration of transgene expression [42]. Minimizing IFN responses could be a useful strategy to increase vaccine potency. This could be achieved by co-administration of compounds able to block IFN responses, like for example vaccinia virus immune evasion proteins [43]. A different approach to boost saRNA vaccines has been based on *in vitro* evolution of RNA replicons in IFN-competent cells [44]. This strategy led to the identification of six mutations in VEEV nonstructural proteins (nsPs) that promoted subgenomic RNA expression. saRNA containing an optimal combination of mutations and expressing interleukin-2 were tested in a murine melanoma murine tumor model, providing enhanced therapeutic activity.

saRNA vaccines for COVID-19

The recent COVID-19 pandemic produced by SARS-CoV-2 has prompted an unprecedented rapid development of many vaccine formulations, including three prototypes based on saRNA. One of them was developed at Imperial College London and is based on VEEV saRNA expressing a pre-fusion stabilized SARS-CoV-2 spike protein encapsulated in LNPs [45••]. This vector induced highly-specific neutralizing antibodies, as well as cellular responses, in mice and is currently being tested in a phase I clinical trial performed with volunteers in the UK. Pfizer

Figure 3
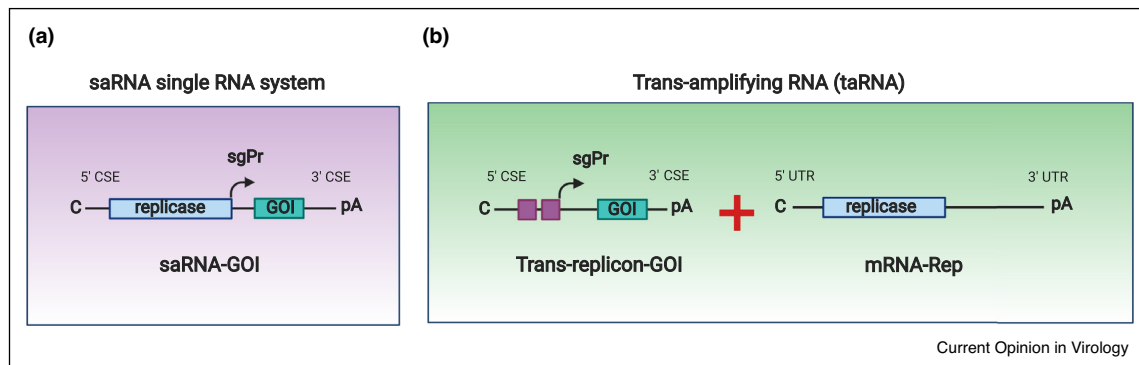
Trans-amplifying RNA (ta-RNA) system. **(a)** saRNA system based on a single saRNA expressing the viral replicase and a gene of interest (GOI). **(b)** Bipartite ta-RNA system based on two RNAs. The first RNA (Trans-replicon-GOI) contains the GOI and the 5′ and 3′ viral conserved sequence elements (CSE) needed for replication. The second RNA (mRNA-Rep) is a non-replicating mRNA expressing the viral replicase. All RNAs are capped (C) and have poly(A) tails (pA). UTR, untranslated regions not involved in replication.

Inc. (New York, NY) in partnership with BioNtech (Germany) has also developed a saRNA prototype vaccine, although the details of this vector/formulation have not been disclosed yet. Finally, a third candidate based on VEEV saRNA expressing SARS-CoV-2 spike encapsulated with Lipid InOrganic Nanoparticles (LION) has been developed by the University of Washington in partnership with HDT Bio Corp. (Seattle, WA) [46••]. This vaccine was able to induce neutralizing antibodies in old mice, as well as in nonhuman primates that lasted for at least 70 days.

Conclusions and future directions

The possibility of using mRNA for vaccination has recently become a reality thanks to the development of highly efficient delivery methods, as the ones described in this review. In particular, combination of these new methodologies with saRNA has demonstrated to be a very efficient way to induce both humoral and cellular immune responses in both rodents and large animal models, being superior to non-replicating mRNA. The reason for this superiority is that transfection with saRNA mimics in many ways a viral infection, inducing a plethora of adjuvant signals that enhance immune responses, without apparent toxicity. One important advantage of saRNA is its versatility, since new vaccines could be generated quickly by changing the sequence coding for the antigen of interest, something that would not affect its production. In fact, saRNA could be efficiently produced at GMP level, using the same technology used to manufacture mRNA. This simplicity and fastness in production could allow generating in a very quick way enough vaccine doses to potentially control emerging viruses that are causing global concern, such as Zika virus, Ebola virus, or the recently appeared SARS-CoV-2.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as

• of special interest
•• of outstanding interest

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