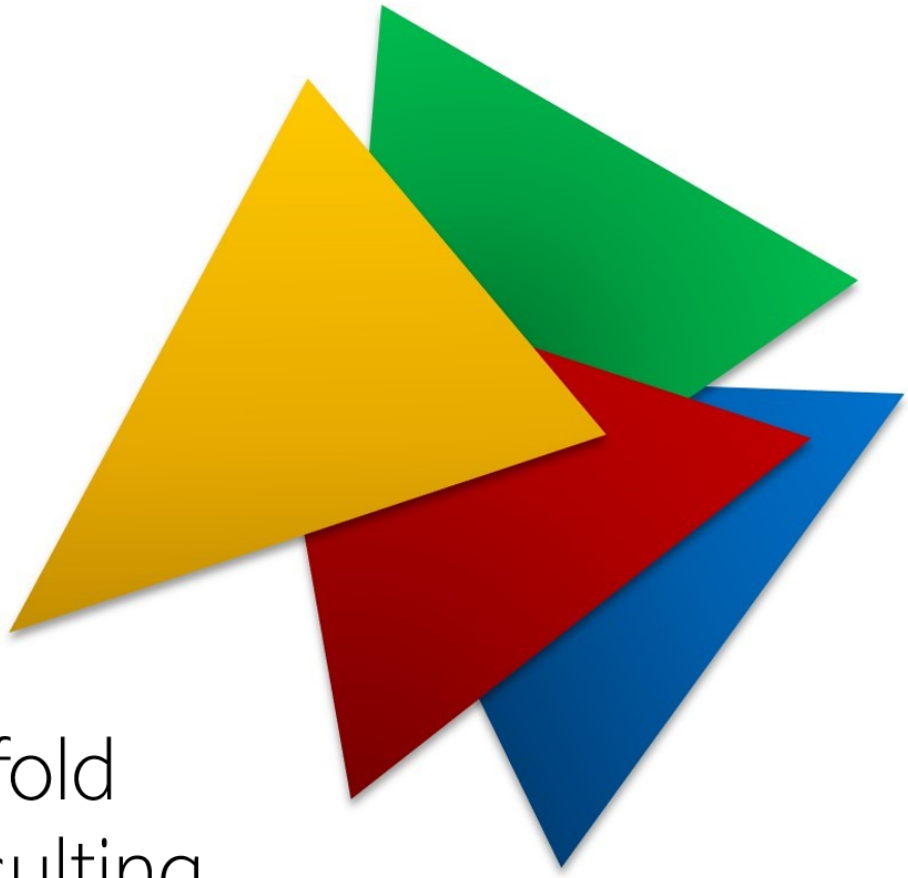
TECHNOLOGY RISK ASSESSMENT

SARS-CoV-2 VACCINE PLATFORMS



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EXECUTIVE SUMMARY

A vaccine is desperately needed to fight the COVID19 pandemic, and a number of candidates are in Phase III clinical trials.
This report details the risks and advantages of new platform technologies, including adenoviral vector-based vaccines and mRNA-based vaccines.

OVERVIEW OF SPECIFIC CONCERNS

An adenoviral-based vaccine against the novel coronavirus, SARS-CoV-2, has now been approved for use in Russia. This has led to suggestions there may be emergency approval of adenoviral-based vaccines in the UK and US. This vaccine involves not only a novel antigen but also a new platform technology.

Adenoviral vector-based products have undergone hundreds of clinical trials over the past 30 years, but have never generated a single licensed product, even for severe diseases with no other treatment options. Now, an adenoviral vector-based gene therapy is being considered for emergency approval for prophylactic use across the entire healthy adult population. A number of scientists have raised specific concerns about the nature of the immune response triggered by adenoviral vector-based vaccines, while others have highlighted the general risk of approving a vaccine prior to the completion of Phase III clinical trials. We advise caution and sustained investigation of potential risks, prior to licensing of this or any new vaccine platform. The risk analysis is presented in full below, but several points are worth emphasizing.

Firstly, due to the nature of adenoviral vector-mediated gene delivery, there is a non-negligible risk of insertional mutagenesis within the host cell genome. This phenomenon has been demonstrated in basic research studies but is sorely under-addressed in the current preclinical and clinical trials for adenoviral vector-based vaccines.

Secondly, due to the nature of adenoviral vector-mediated gene delivery, there is a non-negligible risk of sustained immune response. Preclinical studies in monkeys demonstrated stable expression of the gene encoding MERS coronavirus spike protein for over a year after adenoviral vector-mediated vaccine delivery. This lasting effect may be required for efficacy. However, it may also cause a sustained immune response.

if the vaccine for SARS2 coronavirus spike protein leads to genomic integration or persistent immune system activation in humans, it is important to understand the risks associated with this biological response, and the number of vaccinated patients likely to be affected, since forced expression of the target gene cannot be reversed after the gene therapy is administered. Taking into consideration the nature of this new platform technology, risks of ectopic antigen expression triggered auto-immune disorders and cancers caused by genomic integration of the gene therapy should be evaluated prior to widespread inoculation of the human population with any adenoviral vector-based vaccine.

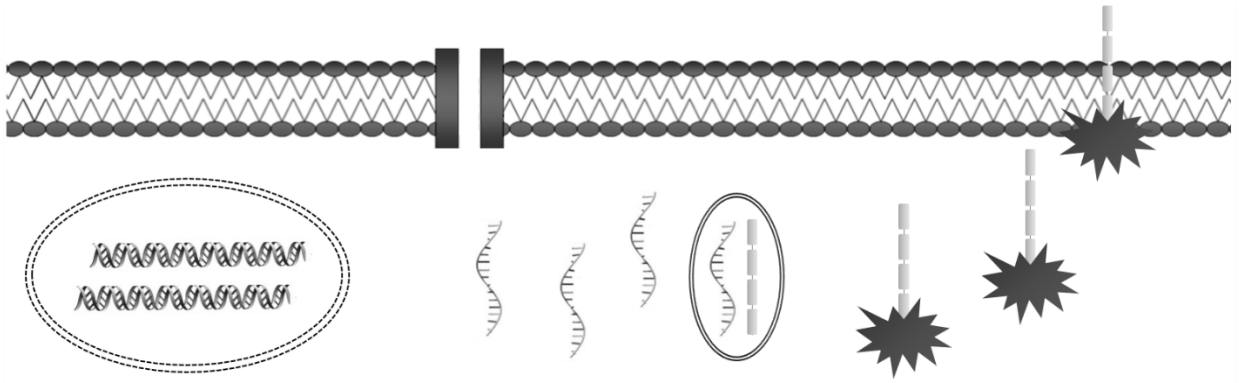
It is not clear these putative risks would occur, but the onus is on the vaccine developer to justify any risks and the government to provide oversight to protect consumers. In this case, it would be advisable to show the rate at which genomic integration occurs, in which regions of the genome and in which cell types, so that frequency of events can be extrapolated to calculate risk across the human population. Likewise, Phase III trials should report any occurrence of debilitating immune response as part of the expected or unexpected adverse effects in those treated with the vaccine. We urge the makers of these new advanced medicinal products to be completely transparent about their preclinical and clinical datasets, so that risks can be accurately assessed. Specifically, data should be shown to support any assertion that adenoviral vector-based vaccines do not cause genomic insertion of encoded genes, germline integration, cancers, auto-immune disorders, or persistent inflammatory conditions. Because this type of vaccine is categorically different from traditional vaccines, running these categorically new risks, and is continuously produced in the body, rather than degraded like proteins or mRNA, it is important to calculate these risks, as the therapy cannot be reversed.

The risks associated with adenoviral vector-based gene therapies have been sufficient to permanently halt clinical trials of this platform technology for proposed use in rare diseases, over the past 30 years, due to safety and efficacy issues. The prospect of approving widespread prophylactic use of this novel platform technology should be considered very carefully, and no shortcuts should be taken in the process of parsing the safety and efficacy profiles of these advanced medicinal products. There is enormous potential for this platform technology, but the nature of each medicinal product, along with its risk-benefit profile, should be fully investigated prior to approval.

If adenoviral vector-mediated vaccines are rolled out in the healthy human population, without proper regard to the risks associated with insertional mutagenesis and prolonged immune system activation, and these concerns prove problematic, there will be a new public health crisis to deal with, even if the approved vaccine has sufficient efficacy and uptake to bring the COVID-19 pandemic under control. A foreseeable catastrophe of this sort will only fuel widespread public mistrust of vaccines, to the detriment of efforts to eradicate disease in the longer term. Therefore, we encourage decision-makers at the FDA, MHRA, and EMA, as well as politicians understandably eager for solutions, to consider risk-benefit profiles of these products carefully, with regard to longer-term outcomes as well as immediate concerns.

BACKGROUND

The novel coronavirus disease, Covid-19, emerged in late 2019 in Wuhan, China, and has unfolded into a worldwide pandemic [1-3]. The pathogen, SARS-CoV-2, was quickly identified with a full genome sequence made available by January 2020 [4-7]. Initially considered to cause 'severe acute respiratory syndrome (SARS)' Covid-19 is now recognized to affect multiple organ systems. Patients present not only with pulmonary distress and low oxygen levels, but also variably with cardiac arrest, arrhythmia, abnormal blood clotting, thrombosis, stroke, encephalitis, anosmia, renal failure, and gastrointestinal issues [8-12]. In some cases, these symptoms are fatal or massively debilitating [13, 14], while in other cases patients remain asymptomatic while spreading the disease [15-18]. Covid-19 has become a dire public health emergency, with a high transmissibility [19-21]. Indeed, there is an urgent need to develop a vaccine, as cases continue to mount and hospitals across many regions exceed bed capacity [22]. In this report, we assess the potential risks of vaccines based on new platform technologies that are currently in late-stage clinical trials. These include adenoviral vector-based vaccines, naked DNA plasmid-based vaccines, and mRNA-based vaccines.



Step 1.	**Step 2.**	**Step 3.**
DNA, which contains the entire genetic code, is *transcribed* in the nucleus. The strands pull apart and one strand is copied to form a single strand of mRNA (messenger RNA).	mRNA floats in the cytoplasm. A localization sequence attracts the strand into the ribosome, where it is *translated* to protein. The protein is then folded into shape and released.	The new protein matches a viral surface protein, also called an antigen. An MHC-I molecule (✹) presents the antigen on the cell surface so the host immune system can learn to attack it.

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Schematic of DNA transcription into mRNA, mRNA translation into proteins, and presentation of proteins as antigens to trigger an immune response. Inoculation trains the body to respond to a viral pathogen in the future. Traditional vaccines deliver inactivated viral proteins. The Moderna and Pfizer platform technologies deliver mRNA into the cell, while the Oxford/AstraZeneca, Johnson & Johnson, Gamaleya Research Institute and CanSino platform technologies rely on delivering DNA encoding the coronavirus spike protein within a modified adenoviral vector.

TRADITIONAL VACCINES

Inoculation with live (attenuated) or killed (inactivated) pathogens has historically been enormously successful in protecting humanity against a number of viral diseases, including smallpox, measles, mumps, rubella, and polio. Yet traditional vaccines against pathogens in the coronavirus family have proven notoriously unsuccessful, as protein derived directly from the viral pathogen does not mount an effective immune response and has been found to cause immunopathogenicity [23-26]. For this reason, researchers have begun to investigate other methods of providing inoculation for SARS-CoV-2 [27]. These methods include delivering genes or messenger RNA encoding select parts of the viral pathogen, particularly surface spike glycoproteins which are known to trigger antibody response [28-30]. In contrast to traditional vaccines, these therapies instruct our own bodies to manufacture antigens similar to proteins found on the surface of the viral pathogen, with cellular presentation of the antigen training the immune response.

ADENOVIRAL VECTOR BASED VACCINES (Oxford/AstraZeneca, Janssen/Johnson & Johnson)

One vaccine type in particular has reached front-runner status, with safety and immunogenicity profiles from Phase I/II clinical trials reported in The Lancet in July 2020 [31-33]. Hopes are high, and large-scale production is already underway so that doses are ready for deployment once Phase III results are complete. This adenoviral vector-based vaccine was designed by researchers at Oxford University, in partnership with the researchers' spin-out company Vaccitech and pharmaceuticals firm AstraZeneca. The group has also partnered with Millipore-Sigma, which is engaging multiple CMOs to manufacture millions of doses, before Phase III trials or challenge trials are complete. A similar product targeting SARS-CoV-2, produced by Janssen in partnership with Johnson & Johnson, is in Phase I trials [34].

These vaccine candidates represent an entirely new approach, based on delivering a viral vector-mediated gene therapy rather than attenuated virus or viral proteins [35]. This is a platform technology that has never previously yielded commercially available product, but it has been put forward as a new method of vaccination for situations in which our bodies do not mount an immune response to attenuated virus or viral proteins [36]. The Oxford vaccine product consists of a gene delivery vehicle, derived from a simian adenovirus, called ChAdOx1. The original adenovirus, which is prevalent in chimpanzees but not humans, was genetically modified to remove the replication machinery from the virus, thereby creating space for new genes to be delivered by the 'gutless' vector [37, 38]. In this region of the viral genome, researchers have inserted a gene encoding the SARS-CoV-2 spike glycoprotein, so the immune system of any person dosed with this therapy will learn to attack cells bearing that antigen [32, 33]. The Janssen vaccine product consists of a gene delivery vehicle derived from a human adenovirus which has low seroprevalence across the global population. This "gutless" vector, derived from adenovirus Ad26, is called AdVac Technology and it can be flexibly engineered to carry antigen-encoding genes [39-42]. This platform is under Phase III investigation in a cohort of individuals at risk of contracting Ebola [43-45].

With any new approach to delivering vaccinations, safety and efficacy must be carefully evaluated prior to administration in the human population. Several issues specific to this new platform technology should be addressed in the course of informing public policy related to this type of vaccine product:

1. Risk of immunogenicity developing against the delivery vector. Human beings have no pre-existing immunity to the Ad5 virus and there is low seroprevalence of Ad26, but after initial dosing with an engineered adenoviral vector, our bodies may learn to mount an immune response to both the preferred antigen and the delivery vehicle. This off-target effect may enhance the immune response to the inoculation [35]. However, this outcome may also render unworkable future ChAdOx1 or Ad26 vaccines made with the same platform, as the immune system has learned to reject the delivery vector. *RISK → low likelihood, low consequence*
2. Risk of off-target effects by components of the vaccine product. Transgene activators are not included in the ChAdOx1 adenoviral vector [38]. These 'helper' genes are normally included to promote gene expression in non-integrating viral vectors, and some of them, such as SV40, are oncogenic [46]. There is no evidence the bovine growth hormone polyadenylation signal sequence (BGH polyA) causes harm to the host cell as it aids mRNA cleavage and export [47]. All sequences in the vector are well-justified. *RISK → mitigated by design*

3. Risk of off-target effects by transduction of non-target cell types. Upon intramuscular or intravenous injection, it is very likely that some non-target cells will be transduced by the adenoviral vector [48, 49]. That may lead to additional cells presenting antigen and being targeted for destruction. Due to the orders of magnitude difference between cell number and number of adenoviral vector particles, there should be no large effect on any single off-target cell population. *RISK → high likelihood, low consequence*
4. Risk of replication competence. ChAdOx1 and Ad26 are replication-deficit adenoviral vectors and should therefore be replication-incompetent [37, 40]. Replication machinery co-opted from other adenoviruses upon concurrent exposure are not known to have suitable flanking regions to permit successful recombination of the genetic material. As a result of this incompatibility, there should be no way for host cells to propagate or replicate the engineered adenoviral vector delivery vehicle. *RISK → low likelihood, medium consequence*
5. Risk of genomic integration. Wild-type adenoviruses do not integrate into the host cell genome, as the genetic material remains in an endosome within the cell and is transcribed from that cytoplasmic location. However, there is some evidence that engineered adenoviral vectors may integrate at titers ranging from 10^10 to 10^13, consistent with the intermediate and high doses used in the ChAdOx1 clinical trials [50]. *RISK → medium likelihood, severe consequence*
6. Risk of dosing. T-cell responses have been shown to accentuate the pathogenesis of Covid-19 by inciting a 'cytokine storm' [51-54]. Any vaccine that constitutively expresses an immune system-triggering antigen may risk causing sustained immunopathogenicity, even in the absence of the SARS-CoV-2 pathogen itself. Long-term effects should be investigated prior to approval. *RISK → medium likelihood, severe consequence*
7. Risk of contamination of the therapeutic product. Biologics, a subset of advanced medicinal products which include cell therapies and gene therapies, cannot be sterilized prior to administration. As a result, they must be manufactured using approved cell lines under highly controlled conditions to prevent contamination and subjected to careful quality control and quality assurance. These measures should not be compromised in an emergency situation [55, 56]. Due to the nature of adenoviral vector products, it is challenging to manufacture large quantities with low risk of contamination. *RISK → medium likelihood, severe consequence*

Overall, there are multiple risks associated with adenoviral vector-based vaccine delivery (Figure 1). Long-term safety and efficacy of these advanced medicinal products should be investigated prior to approval for use in humans, with careful attention to the specific risks cited here. It should be noted with regard to this therapeutic approach that there is no precedent for the licensing of an engineered viral vector vaccine in the history of public health. Clinical trials for vaccines against HIV and Ebola, using similar adenoviral vector platform technologies, have been previously halted due to efficacy issues [57-61]. The ChAdOx1 platform is new, and the Ad26 platform vaccine against HIV, Ad26-ENVA-01, is now being evaluated in combination with an MVA adjuvant [62]. This is a largely unproven gene therapy approach, with no fully licensed products to-date and a long history of clinical trial failures over the past twenty years [36, 62]. The risks should be carefully considered prior to rolling out any vaccines based on this platform technology.

DNA BASED VACCINES (Inovio)

Another method of gene delivery into cells, for the express purpose of encoding a viral antigen, involves injecting double-stranded DNA without a viral vector-mediated delivery system. The genetic material must get into the host cell; this can be achieved by electrically disrupting the cell membrane.

This technology has been pioneered by the company Inovio [63, 64], which has developed double-stranded DNA vaccines for HPV (VGX-3100), HIV (PENNVAX-GP), Ebola (INO-4201), Zika (INO-4600), MERS (INO-4700) and SARS-CoV-2 (INO-4800); each vaccine has shown promise in animal studies [65-70]. The company is currently conducting Phase I/II trials for these disease indications [71, 72] and has begun a Phase III trial for the HPV vaccine, after earlier trials demonstrated clearance of HPV-induced dysplasia [66, 73, 74]. This prophylactic approach requires the use of a calibrated, proprietary electroporation device, called CELLECTRA®, to force double-stranded DNA into cells after the plasmids are delivered by injection [75-78].

From the company website: "The CELLECTRA device uses a brief electrical pulse to reversibly open small pores in the cell to allow plasmids to enter, overcoming a key limitation of other DNA and other nucleic acid approaches, such as mRNA. Once inside the cell, the DNA plasmids enable the cell to produce the targeted antigen. The antigen is processed naturally in the cell and triggers the desired T cell and antibody-mediated immune responses." Indeed, animal studies have demonstrated the stimulation of strong immune responses, with increased antibody titers and T cell activity, in response to DNA-based vaccines built on this platform technology [65-70]. While the efficacy of the method has strong support in multiple studies, safety questions with regard to electroporation remain [71, 72].

With any new approach to delivering vaccinations, safety and efficacy must be carefully evaluated prior to administration in the human population. Issues specific to this new platform technology should be addressed prior to deciding public health policy related to this potential vaccine:

1. Risk of the delivery method. Electroporation is commonly used to transduce cells in culture, for research purposes, as the electrical pulse can be spread evenly across cells in suspension. The approach of electrically disrupting the cellular membrane to achieve this goal is not considered a reliable method of gene delivery into a live organism, in either a research or clinical setting, and it is an gene therapy method that carries its own safety risk despite the absence of a viral vector-mediated delivery system. If this were a reliable method, then researchers would not have gone through the trouble of taking apart entire viral vectors, gutting the replication machinery, and designing them to carry a genetic payload. Viruses have evolved over hundreds of millions of years to effectively deliver their genetical material into host cells, and engineered viral vectors take advantage of this biological complexity. It is difficult to deliver DNA into cells without exploiting these natural strategies. The acute and long-term safety issues associated with disrupting cell membranes across a region of tissue *in vivo* in order to force double-stranded DNA into cells are not well-understood; more studies investigating the post-electroporation histology of dermal and muscular tissues are needed to assess this risk. Published work focuses on immune response and cytotoxicity, which are demonstrable. *RISK* → *uncertain*

2. Risk of off-target effects by components of the vaccine product. The genetic sequence is proprietary and not available for public consideration, although early papers from the company mention codon optimization, cytoplasmic tail truncation and additional sequences to enhance transcription and translation [70, 79]. It is reasonable to expect regulators to take a close look at the sequence and ensure it does not contain oncogenic transgene activators or other DNA targeting sequences. Inovio has begun adding an adjuvant to its vaccines, a double-stranded DNA plasmid encoding IL-12, called INO-9012; this pro-inflammatory cytokine triggers a stronger immune response [80]. In addition to the acute damage caused by electroporation, the presence of double-stranded DNA in the bloodstream is itself reactive, as this signals to the immune system that cells may have been destroyed in the vicinity, thereby triggering an immune response. *RISK → uncertain*
3. Risk of off-target effects by transduction of non-target cell types. The method of using electroporation to deliver double-stranded DNA should not lead to a significant effect on any single off-target cell population. 92% of patients in the INO-4700 clinical trial reported pain at the site of administration, indicating local tissue damage; about a third reported headaches after receiving the vaccine, indicating a broad, systemic response. However, most patients in each dosing group reported these symptoms to be mild [71]. *RISK → uncertain*
4. Risk of replication competence. Plasmids containing double-stranded DNA will not contain replication machinery, although this genetic material can be replicated along with the host cell DNA after nuclear entry and genomic integration. There can be no viral propagation without any delivery vector; therefore, so long as germ cells are not transduced, there should be no risk of passing the DNA onward. *RISK → mitigated by design*
5. Risk of genomic integration. Plasmids containing double-stranded DNA persist in cytoplasmic endosomes after injection; electroporation disrupts both the plasma membrane and the nuclear envelope so plasmids can gain entry to the nucleus and integrate into the host cell genome [81-84]. This allows persistent gene expression of the target material; however, this event also increases risks of insertional mutagenesis. Insertion rate and insertion sites should be investigated prior to approval. *RISK → high likelihood, medium consequence*
6. Risk of dosing. T-cell responses have been shown to accentuate the pathogenesis of Covid-19 by inciting a 'cytokine storm' [51-54]. A DNA-based vaccine that constitutively expresses an immune-triggering antigen and immune-triggering adjuvants may risk causing a sustained immune reaction, even in the absence of SARS-CoV-2 itself. If the double-stranded DNA successfully integrates into the host cell genome to achieve transcription, dosing will be controlled to some extent by transcriptional activity within the host cell and expression may remain constitutive. Protein expression after this method of gene delivery has been shown to be sustained for at least two weeks in rodents [85] and one year in monkeys [68]. The effects of sustained immunopathogenicity on overall health should be investigated prior to approval. *RISK → uncertain*
7. Risk of contamination of the therapeutic product. Biologics, a subset of advanced medicinal products which include cell therapies and gene therapies, cannot be sterilized prior to administration. However it is easy to manufacture large quantities of double-stranded DNA at high purity with low risk of contamination. Yet infections in a fraction of patients dosed with the INO-4700 vaccine should trigger a closer investigation of manufacturing processes and quality control practices. *RISK → medium likelihood, medium consequence*

Overall, there are several safety risks associated with DNA based vaccines (Figure 2). These risks relate to both the product itself and the electroporation delivery method. The acute and longer-term safety profile of these advanced medicinal products should be investigated prior to widespread use in the human population, and the effectiveness of this prophylaxis should be balanced against the potential risk of tissue damage due to electroporation, the potential risk of insertional mutagenesis, and the potential risk of adverse response caused by exposure to the double-stranded DNA, the gene products themselves, any adjuvants included in the therapy, and any additional DNA or gene products amassed as a result of the therapy. It is recommended that longer-term histopathological results are explored in animal studies for this vaccine platform, and that early-phase clinical trials address the effects of electroporating double-stranded DNA products into tissue, using techniques that go beyond verbal reporting of adverse effects.

mRNA BASED VACCINES (Pfizer/BioNTech and Moderna)

Another vaccine platform has reached front-runner status, with two groups reporting progress in Phase I/II clinical trials in July 2020 [86-90]. Two mRNA vaccine candidates have been developed by Pfizer and BioNTech (BNT-162b1 and BNT-162b2). One mRNA vaccine candidate against SARS-CoV-2 has been developed by Moderna (mRNA-1273). These companies have already been contracted to manufacture millions of doses before Phase III trials are complete.

This vaccine is an entirely new approach, based on delivering messenger RNA (mRNA) rather than attenuated virus or viral proteins. It is a platform technology that has never previously yielded commercially available product. The vaccine consists of a single-stranded mRNA molecule encoding a prefusion stabilized form of SARS-CoV-2 spike glycoprotein, encased in lipids [91, 92]. The coated mRNA is taken up by cells in the inoculated individual; the mRNA makes its way to the ribosome where it is translated into protein. The antigen is then presented on the presenting cell surface so the immune system of any person dosed with this therapy learns to attack that antigen.

Moderna reported results from Phase I trials on July 14 and began Phase III trials on July 27 [87, 88]. Patients were given two inoculations of mRNA-1273, 28 days apart, at doses of 25 μg, 100 μg, or 250 μg (n=15/group). Results from this initial study demonstrated dose-dependent antibody titers, with over half the participants reporting adverse events like fatigue, headache, myalgia, and pain at the injection site. Phase III trials will move ahead with the 100 μg dose.

Pfizer reported results from Phase I/II trials for BNT162b1 in a non-peer-reviewed study published July 1 on medrxiv. They have not published any results for BNT162b2 prior to advancing the latter product to Phase III trials on July 27 [93]. In Phase I/II trials, patients were given two inoculations of BNT162b1, 21 days apart, at doses of 10 μg, 30 μg, or 100 μg (n=12/group, plus 9 placebo-treated individuals). Patients in the low and medium dose groups had significantly elevated antibody titer at day 28, with no serious adverse events, although over 80% of these patients reported pain at the injection site (compared with 16.7% of the placebo group). In the same clinical trial, Pfizer reports on its website that 100+ patients were given two 30 μg doses of mRNA vaccine product BNT162b2, which demonstrated a 'favorable overall tolerability profile' with generally mild to moderate adverse events such as fever, chills, and fatigue. The company also touts robust T cell activation and increased titers of neutralizing antibodies for the SARS-CoV-2 spike glycoprotein. Yet these data remain unpublished, as this candidate vaccine product BNT162b2 moves into Phase III trials.

With any new approach to delivering vaccinations, safety and efficacy must be carefully evaluated prior to administration in the human population. Although many potential risks are mitigated by design, issues specific to this new platform technology should be addressed prior to deciding public health policy related to mRNA vaccines:

1. Risk of the delivery method. Both Moderna and Pfizer/BioNTech mRNA products are encapsulated in lipid nanoparticles to facilitate cell entry; there is no vector or electroporation [91]. *RISK* → *mitigated by design*

2. **Risk of off-target effects by components of the vaccine product.** No transgene activators are required for the function of an mRNA product. Both sequences are lean and codon-optimized, and engineered to have ideal nucleoside modification in the case of the Pfizer/BioNTech product [94]. *RISK → mitigated by design*
3. **Risk of off-target effects by transduction of non-target cell types.** Upon vaccine delivery, it is likely that some non-target cells will be transduced by the mRNA product. Due to the orders of magnitude difference between cell number and number of encapsulated mRNA particles, there should not be a significant effect on any single off-target cell population. *RISK → high likelihood, low consequence*
4. **Risk of replication competence.** mRNA is replication-incompetent nucleic acid material and survives within the cell only long enough to be translated into protein [95]. Another Pfizer/BioNTech candidate sequence is a self-amplifying mRNA, but it is not being put into clinical trial at this time. *RISK → mitigated by design*
5. **Risk of genomic integration.** There is no risk for integration of mRNA into the host cell genome. mRNA products may contain a localization sequence which targets the strand to the ribosome, but this material will not gain entry to the cell nucleus and has no mechanism for integration [96]. *RISK → mitigated by design*
6. **Risk of dosing.** T-cell responses have been shown to accentuate the pathogenesis of Covid-19 by inciting a 'cytokine storm' [51-54]. An mRNA-based vaccine that expresses an immune-triggering antigen for some weeks may risk causing immunopathogenicity, even in the absence of SARS-CoV-2 itself. Due to the quick mRNA degradation process, this should be a short-lived effect. *RISK → low likelihood, low consequence*
7. **Risk of contamination of the therapeutic product.** Biologics, a subset of advanced medicinal products which include cell therapies and gene therapies, cannot be sterilized prior to administration. Yet due to the simple, lean nature of mRNA products, it is easy to manufacture large quantities at high purity with low risk of contamination. Without the need to manufacture product in cell lines, the risks of contamination and types of potential contamination for mRNA vaccines are low. *RISK → low likelihood, low consequence*

Overall, there are few safety risks anticipated with mRNA based vaccine delivery (Figure 3). The product contains no delivery vector that could itself trigger an immune response. mRNA cannot replicate and cannot integrate into the host cell genome. The mRNA product is not very stable and is unlikely to last very long in the bloodstream or in cells, thereby providing a short window of inoculation; however, this feature also presents challenges with dosing. With regard to ease of manufacture, this product can be made in high quantities, at high purity, for very low cost. Although the nature of the medicinal product provides little cause for concern, the long-term safety and efficacy of these advanced medicinal products should be investigated prior to widespread use in the human population. The primary safety concerns, and optimal dosing to achieve expression of viral antigens in target immune cells, have been addressed in Phase I/II trials. The question of efficacy, with regard to the protection provided against SARS-CoV-2 by these new mRNA vaccines, will be evaluated in the Phase III trials soon to be underway.

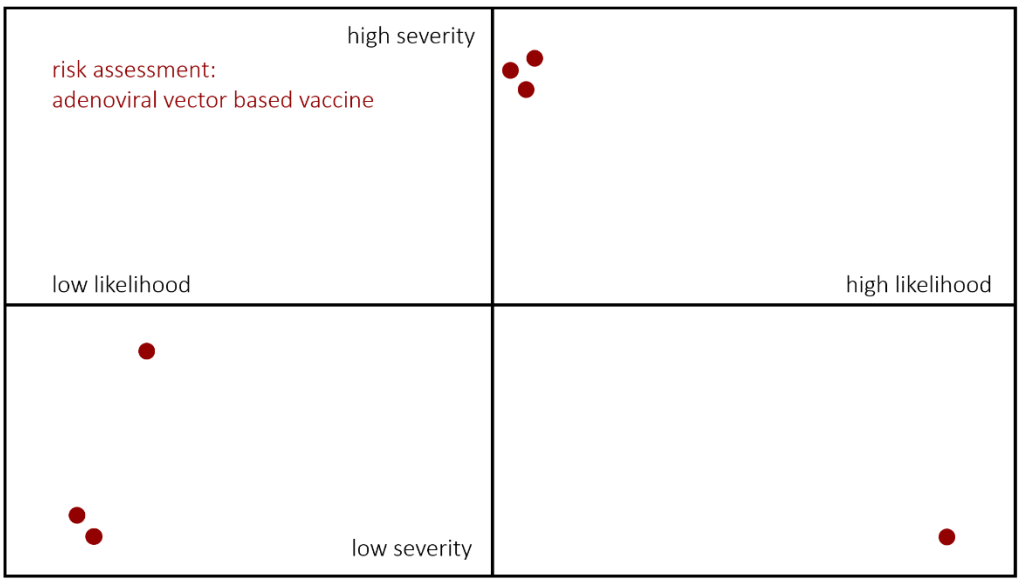

Figure 1. Qualitative risk assessment on adenoviral vaccines. Based on risks identified in the document text, a number of potential issues with this platform technology are flagged, with additional safety studies being recommended prior to approval for use in humans, particularly the risk of genomic insertion and auto-immune response.

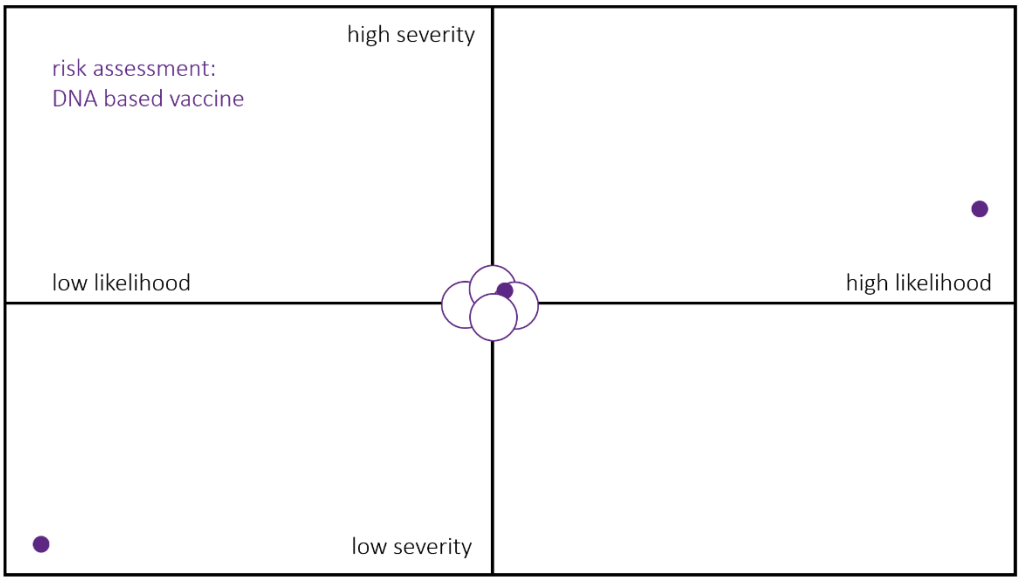

Figure 2. Qualitative risk assessment on DNA based vaccines. Based on risks identified in the document text, a number of potential issues with this platform technology are flagged as uncertain, particularly in relation to effects of electroporation on local cell populations. Additional safety studies are recommended prior to approval for use in humans.

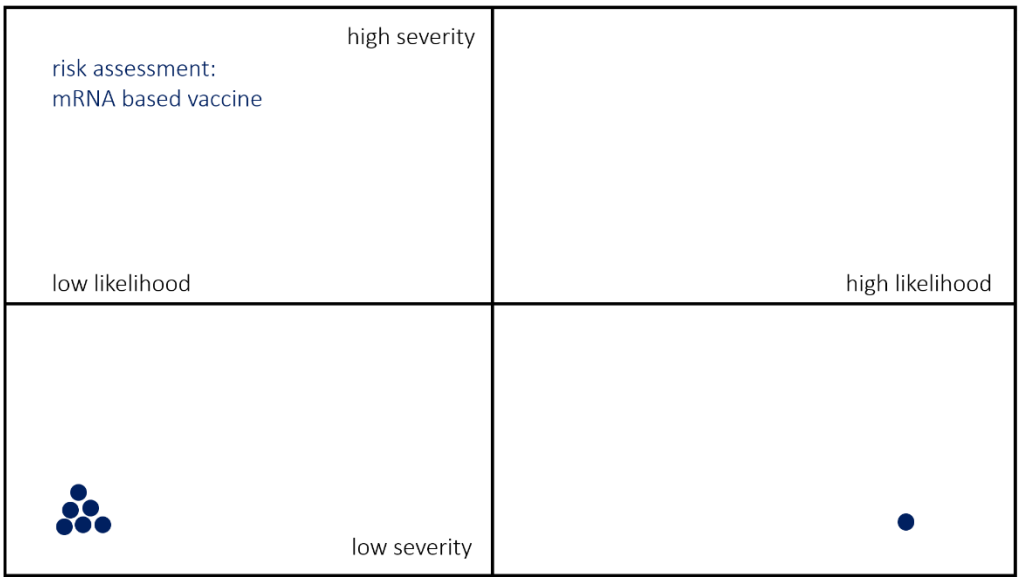

Figure 3. Qualitative risk assessment on mRNA vaccines. Based on risks identified in the document text, few potential issues are foreseen with this vaccine platform technology. However shortcuts in safety and efficacy studies are not recommended prior to approval for use in humans.

CONCLUSIONS

We at Conifold Consulting Services are rooting for success in the search for a vaccine to protect humanity against the novel coronavirus. Like many others, we are enthusiastic about the prospects of new technology being developed to fight pathogens. However, we encourage proper evaluation of new advanced therapeutics before these are approved for use in humans. With hands-on professional experience in the design, manufacture, and validation of vector-based gene therapies, we certainly advocate for the promise of this technology in otherwise-intractable diseases. However, advanced medicinal products, particularly biologicals that are based on new platform technologies, must be rigorously tested with careful oversight. Decision-makers must carefully weigh the urgency of the immediate situation and the potential benefits of a new vaccine platform in the short term over the potential long-term risks of these therapies and the critical importance of maintaining public trust in the approvals process. It must be acknowledged that every public health and governing body in the world has a conflict of interest in assessing risk-reward right now, with a strong desire to end the pandemic as soon as possible. We urge those in decision-making roles to remain aware of this cognitive bias, and to carefully consider the risks of rolling out an entirely new vaccine platform to the US population without adequate discussion of possible hazards.

A NOTE ON PUBLIC HEALTH MESSAGING

Public health messaging during this pandemic has proven challenging in the context of public hesitancy fueled by misinformation campaigns. The WHO and CDC have provided concise and useful public health advice with regard to social distancing and handwashing. It is now appropriate to begin messaging with regard to vaccination, in preparation for the eventuality of having a safe and effective vaccine against COVID-19.

Concerned individuals with little training in the biological sciences may benefit greatly from public health messaging that focuses not only on what they can do to keep safe, but also on the personal responsibility of public health officials and medical professionals. With low levels of trust running through our society, the public needs to be assured that scientists and doctors and policymakers are indeed rigorously examining the safety and effectiveness of any vaccines or therapeutics. Firstly, discussing a commitment to public health as a personal calling is a messaging strategy which allows members of the public to connect with professionals in the healthcare field. Secondly, engaging deeply with the difficult questions will also aid in building trust with the community at large. And thirdly, communicating the results of basic research studies and clinical trials, with an emphasis on oversight by independent observers, will help members of the public to support decisions based on risk-benefit analysis.

Some messaging prompts include:

- We were lucky to avoid a pandemic with the emergence of MERS a decade ago and SARS nearly two decades ago. We were not so lucky this time, as “SARS2” aka “COVID-19” – the novel coronavirus – has unfolded into a worldwide pandemic.
- Coronaviruses and other pathogens pose a great threat to humanity. As much as we would prefer not to believe this is happening, denial will not protect us from the virus. What will protect us is wearing a mask, standing six feet apart, and making sure to wash our hands regularly.
- “Treatments save lives, but vaccines save populations.” When you get a vaccine, you keep yourself safe – but you also keep your loved ones safe. Vaccines stop the chain of transmission. If you have been inoculated with a vaccine, you will not pass that disease onwards.
- A vaccine takes a little bit of the wild coronavirus and introduces our bodies to that biological material. Once our immune systems are familiar with those characteristic spikes of the coronavirus, our bodies are trained to respond and fight when the real thing comes along. Inoculation protects us in advance, by preparing our bodies to recognize and neutralize the coronavirus.
- It is very important that we test the safety and efficacy of any new vaccines. Public health officials take responsibility for ensuring that *any* new therapies or preventative medicines, including vaccines, always do more good than harm.

REFERENCES:

1. Cohen, J. and D. Normile, *New SARS-like virus in China triggers alarm.* Science, 2020. 367(6475): p. 234-235.
2. Cucinotta, D. and M. Vanelli, *WHO Declares COVID-19 a Pandemic.* Acta Biomed, 2020. 91(1): p. 157-160.
3. Zhou, P., et al., *A pneumonia outbreak associated with a new coronavirus of probable bat origin.* Nature, 2020. 579(7798): p. 270-273.
4. Lu, R., et al., *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.* Lancet, 2020. 395(10224): p. 565-574.
5. Kim, D., et al., *The Architecture of SARS-CoV-2 Transcriptome.* Cell, 2020. 181(4): p. 914-921 e10.
6. Gussow, A.B., et al., *Genomic determinants of pathogenicity in SARS-CoV-2 and other human coronaviruses.* Proc Natl Acad Sci U S A, 2020. 117(26): p. 15193-15199.
7. Lam, T.T., et al., *Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins.* Nature, 2020. 583(7815): p. 282-285.
8. Gupta, A., et al., *Extrapulmonary manifestations of COVID-19.* Nat Med, 2020. 26(7): p. 1017-1032.
9. Kwenandar, F., et al., *Coronavirus disease 2019 and cardiovascular system: A narrative review.* Int J Cardiol Heart Vasc, 2020. 29: p. 100557.
10. Lodigiani, C., et al., *Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy.* Thromb Res, 2020. 191: p. 9-14.
11. Qureshi, A.I., et al., *Management of acute ischemic stroke in patients with COVID-19 infection: Report of an international panel.* Int J Stroke, 2020. 15(5): p. 540-554.
12. Palmieri, L., et al., *Clinical Characteristics of Hospitalized Individuals Dying with COVID-19 by Age Group in Italy.* J Gerontol A Biol Sci Med Sci, 2020.
13. Goldman, I.A., K. Ye, and M.H. Scheinfeld, *Lower extremity arterial thrombosis associated with COVID-19 is characterized by greater thrombus burden and increased rate of amputation and death.* Radiology, 2020: p. 202348.
14. Varatharaj, A., et al., *Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study.* Lancet Psychiatry, 2020.
15. Day, M., *Covid-19: four fifths of cases are asymptomatic, China figures indicate.* BMJ, 2020. 369: p. m1375.
16. Arons, M.M., et al., *Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility.* N Engl J Med, 2020. 382(22): p. 2081-2090.
17. Gostic, K., et al., *Estimated effectiveness of symptom and risk screening to prevent the spread of COVID-19.* Elife, 2020. 9.
18. Koo, J.R., et al., *Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study.* Lancet Infect Dis, 2020. 20(6): p. 678-688.
19. Mahase, E., *Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate.* BMJ, 2020. 368: p. m641.
20. Signorelli, C., et al., *COVID-19 mortality rate in nine high-income metropolitan regions.* Acta Biomed, 2020. 91(9-S): p. 7-18.
21. Wang, Y., et al., *Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures.* J Med Virol, 2020. 92(6): p. 568-576.
22. Tanne, J.H., *Covid-19: US sees record rise in cases.* BMJ, 2020. 370: p. m2676.
23. Roper, R.L. and K.E. Rehm, *SARS vaccines: where are we?* Expert Rev Vaccines, 2009. 8(7): p. 887-98.
24. Tseng, C.T., et al., *Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.* PLoS One, 2012. 7(4): p. e35421.
25. Agrawal, A.S., et al., *Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.* Hum Vac Immunother, 2016. 12(9): p. 2351-6.
26. Enjuanes, L., et al., *Molecular Basis of Coronavirus Virulence and Vaccine Development.* Adv Virus Res, 2016. 96: p. 245-286.
27. Chen, W.H., et al., *The SARS-CoV-2 Vaccine Pipeline: an Overview.* Curr Trop Med Rep, 2020: p. 1-4.
28. Poh, C.M., et al., *Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients.* Nat Commun, 2020. 11(1): p. 2806.
29. Lan, J., et al., *Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.* Nature, 2020. 581(7807): p. 215-220.

30. Walls, A.C., et al., *Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein.* Cell, 2020. 181(2): p. 281-292 e6.
31. Gallagher, J., *Coronavirus: Oxford vaccine triggers immune response.* BBC World News https://www.bbc.com/news/uk-53469839, 2020.
32. Folegatti, P.M., et al., *Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: a dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial.* Lancet Infect Dis, 2020. 20(7): p. 816-826.
33. Folegatti, P.M., et al., *Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial.* Lancet, 2020.
34. Carlson, R., *Ad26.COV2-S SARS-CoV-2 Vaccine.* Precision Vaccinations https://www.precisionvaccinations.com/vaccines/ad26cov2-s-sars-cov-2-vaccine, 2020.
35. Lee, C.S., *Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine.* Genes Dis, 2017. 4(2): p. 43–63.
36. Cross, R., *Adenoviral vectors are the new COVID-19 vaccine front-runners. Can they overcome their checkered past? .* Chemical & Engineering News https://cen.acs.org/pharmaceuticals/vaccines/Adenoviral-vectors-new-COVID-19/98/i19, 2020. 98(19): p. 20-23.
37. Dicks, M.D., et al., *A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity.* PLoS One, 2012. 7(7): p. e40385.
38. Morris, S.J., et al., *Simian adenoviruses as vaccine vectors.* Future Virology, 2016. 11(9): p. 649–659.
39. Barouch, D.H., et al., *Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys.* Science, 2015. 349(6245): p. 320-4.
40. Geisbert, T.W., et al., *Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge.* J Virol, 2011. 85(9): p. 4222-33.
41. Teigler, J.E., M.J. Iampietro, and D.H. Barouch, *Vaccination with adenovirus serotypes 35, 26, and 48 elicits higher levels of innate cytokine responses than adenovirus serotype 5 in rhesus monkeys.* J Virol, 2012. 86(18): p. 9590-8.
42. Zahn, R., et al., *Ad35 and ad26 vaccine vectors induce potent and cross-reactive antibody and T-cell responses to multiple filovirus species.* PLoS One, 2012. 7(12): p. e44115.
43. Kimbugwe, G., *An Open-label, Single Arm Study to Provide Additional Information on Immunogenicity and Safety of Ad26.ZEBOV/MVA-BN-Filo.* Clinical Trials https://clinicaltrials.gov/ct2/show/record/NCT04028349, 2019.
44. Kavunga, H., *Single arm, open-label, non-randomized interventional trial of the two dose, Ad26.ZEBOV, MVA-BN-Filo Ebola preventative vaccine.* Clinical Trials https://clinicaltrials.gov/ct2/show/NCT04152486, 2019.
45. World Health Organization, *Update with the development of Ebola vaccines and implications of emerging evidence to inform future policy recommendations.* https://www.who.int/immunization/sage/meetings/2018/october/2_Ebola_SAGE2018Oct_BgDoc_20180919.pdf, 2018.
46. Weiss, R., et al., *SV40 as an oncogenic virus and possible human pathogen.* Dev Biol Stand, 1998. 94: p. 355-60, 369-82.
47. Ren, X.G., et al., *Construction of a recombinant BHV-1 expressing the VP1 gene of foot and mouth disease virus and its immunogenicity in a rabbit model.* Biotechnol Lett, 2009. 31(8): p. 1159-65.
48. Kalesnykas, G., et al., *Comparative Study of Adeno-associated Virus, Adenovirus, Bacu lovirus and Lentivirus Vectors for Gene Therapy of the Eyes.* Curr Gene Ther, 2017. 17(3): p. 235-247.
49. Kuhlmann, K.F., D.J. Gouma, and J.G. Wesseling, *Adenoviral gene therapy for pancreatic cancer: where do we stand?* Dig Surg, 2008. 25(4): p. 278-92.
50. Stephen, S.L., et al., *Chromosomal integration of adenoviral vector DNA in vivo.* J Virol, 2010. 84(19): p. 9987-94.
51. Chiappelli, F., A. Khakshooy, and G. Greenberg, *CoViD-19 Immunopathology and Immunotherapy.* Bioinformation, 2020. 16(3): p. 219-222.
52. Codo, A.C., et al., *Elevated Glucose Levels Favor SARS-CoV-2 Infection and Monocyte Response through a HIF-1alpha/Glycolysis-Dependent Axis.* Cell Metab, 2020.

53. Zhang, X., et al., *Viral and host factors related to the clinical outcome of COVID-19.* Nature, 2020. 583(7816): p. 437-440.
54. Nicholls, J.M., et al., *Lung pathology of fatal severe acute respiratory syndrome.* Lancet, 2003. 361(9371): p. 1773-8.
55. Gandara, C., V. Affleck, and E.A. Stoll, *Manufacture of Third-Generation Lentivirus for Preclinical Use, with Process Development Considerations for Translation to Good Manufacturing Practice.* Hum Gene Ther Methods, 2018. 29(1): p. 1-15.
56. White, M., et al., *A Guide to Approaching Regulatory Considerations for Lentiviral-Mediated Gene Therapies.* Hum Gene Ther Methods, 2017. 28(4): p. 163-176.
57. Baden, L.R., et al., *First-in-Human Randomized, Controlled Trial of Mosaic HIV-1 Immunogens Delivered via a Modified Vaccinia Ankara Vector.* J Infect Dis, 2018. 218(4): p. 633-644.
58. Baden, L.R., et al., *First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001).* J Infect Dis, 2013. 207(2): p. 240-7.
59. Barouch, D.H., et al., *Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19).* Lancet, 2018. 392(10143): p. 232-243.
60. Mast, T.C., et al., *International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials.* Vaccine, 2010. 28(4): p. 950-7.
61. Matz, K.M., A. Marzi, and H. Feldmann, *Ebola vaccine trials: progress in vaccine safety and immunogenicity.* Expert Rev Vaccines, 2019. 18(12): p. 1229-1242.
62. Pitisuttithum, P. and M.A. Marovich, *Prophylactic HIV vaccine: vaccine regimens in clinical trials and potential challenges.* Expert Rev Vaccines, 2020. 19(2): p. 133-142.
63. [Anonymous], *The Race to Find a COVID-19 Vaccine: The INO-4800 Study Participation at the University of Pennsylvania.* Trial Site News https://www.trialsitenews.com/the-race-to-find-a-covid-19-vaccine-the-ino-4800-study-participation-at-the-university-of-pennsylvania/, 2020.
64. [Anonymous], *Government-Supported Inovio Sued for Alleged Stock Manipulation.* Trial Site News https://www.trialsitenews.com/government-supported-inovio-sued-for-alleged-stock-manipulation/, 2020.
65. Griffin, B.D., et al., *DNA vaccination protects mice against Zika virus-induced damage to the testes.* Nat Commun, 2017. 8: p. 15743.
66. Morrow, M.P., et al., *Augmentation of cellular and humoral immune responses to HPV16 and HPV18 E6 and E7 antigens by VGX-3100.* Mol Ther Oncolytics, 2016. 3: p. 16025.
67. Muthumani, K., et al., *A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates.* Sci Transl Med, 2015. 7(301): p. 301ra132.
68. Patel, A., et al., *Protective Efficacy and Long-Term Immunogenicity in Cynomolgus Macaques by Ebola Virus Glycoprotein Synthetic DNA Vaccines.* J Infect Dis, 2019. 219(4): p. 544-555.
69. Smith, T.R.F., et al., *Immunogenicity of a DNA vaccine candidate for COVID-19.* Nat Commun, 2020. 11(1): p. 2601.
70. Yan, J., et al., *Immunogenicity of a novel engineered HIV-1 clade C synthetic consensus-based envelope DNA vaccine.* Vaccine, 2011. 29(41): p. 7173-81.
71. Modjarrad, K., et al., *Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial.* Lancet Infect Dis, 2019. 19(9): p. 1013-1022.
72. Tebas, P., et al., *Safety and Immunogenicity of an Anti-Zika Virus DNA Vaccine - Preliminary Report.* N Engl J Med, 2017.
73. Hollenberg, R.K., et al., *Safety and immunogenicity of VGX-3100 formulations in a healthy young adult population.* Hum Vaccin Immunother, 2020. 16(6): p. 1404-1412.
74. Trimble, C.L., et al., *Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial.* Lancet, 2015. 386(10008): p. 2078-2088.
75. Broderick, K.E., et al., *Prototype development and preclinical immunogenicity analysis of a novel minimally invasive electroporation device.* Gene Ther, 2011. 18(3): p. 258-65.

76. Lin, F., et al., *Optimization of electroporation-enhanced intradermal delivery of DNA vaccine using a minimally invasive surface device.* Hum Gene Ther Methods, 2012. 23(3): p. 157-68.
77. Lin, F., et al., *A novel prototype device for electroporation-enhanced DNA vaccine delivery simultaneously to both skin and muscle.* Vaccine, 2011. 29(39): p. 6771-80.
78. Diehl, M.C., et al., *Tolerability of intramuscular and intradermal delivery by CELLECTRA((R)) adaptive constant current electroporation device in healthy volunteers.* Hum Vaccin Immunother, 2013. 9(10): p. 2246-52.
79. Cashman, K.A., et al., *Enhanced Efficacy of a Codon-Optimized DNA Vaccine Encoding the Glycoprotein Precursor Gene of Lassa Virus in a Guinea Pig Disease Model When Delivered by Dermal Electroporation.* Vaccines (Basel), 2013. 1(3): p. 262-77.
80. Aggarwal, C., et al., *Immune Therapy Targeting E6/E7 Oncogenes of Human Paillomavirus Type 6 (HPV-6) Reduces or Eliminates the Need for Surgical Intervention in the Treatment of HPV-6 Associated Recurrent Respiratory Papillomatosis.* Vaccines (Basel), 2020. 8(1).
81. Bai, H., et al., *Cytoplasmic transport and nuclear import of plasmid DNA.* Biosci Rep, 2017. 37(6).
82. Cervia, L.D., et al., *Enhancing Electrotransfection Efficiency through Improvement in Nuclear Entry of Plasmid DNA.* Mol Ther Nucleic Acids, 2018. 11: p. 263-271.
83. Ledwith, B.J., et al., *Plasmid DNA vaccines: assay for integration into host genomic DNA.* Dev Biol (Basel), 2000. 104: p. 33-43.
84. Wang, Z., et al., *Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation.* Gene Ther, 2004. 11(8): p. 711-21.
85. Mendoza, J.M., et al., *Elucidating the Kinetics of Expression and Immune Cell Infiltration Resulting from Plasmid Gene Delivery Enhanced by Surface Dermal Electroporation.* Vaccines (Basel), 2013. 1(3): p. 384-97.
86. Akpan, N., *Moderna's mRNA vaccine reaches its final phase. Here's how it works.* National Geographic https://www.nationalgeographic.com/science/2020/05/moderna-coronavirus-vaccine-how-it-works-cvd/ 2020.
87. Jackson, L.A., *An mRNA Vaccine against SARS-CoV-2 — Preliminary Report.* N Engl J Med, 2020: p. NEJMoa2022483.
88. Release], M.P., *Moderna Announces Phase 3 COVE Study of mRNA Vaccine Against COVID-19 (mRNA-1273) Begins.* https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-phase-3-cove-study-mrna-vaccine-against-covid, 2020.
89. Release], B.P., *Pfizer and BioNTech Announce Early Positive Data from an Ongoing Phase 1/2 Study of mRNA-based Vaccine Candidate Against SARS-CoV-2.* https://investors.biontech.de/news-releases/news-release-details/pfizer-and-biontech-announce-early-positive-data-ongoing-phase, 2020.
90. Release], P.P., *Pfizer and BioNTech Choose Lead mRNA Vaccine Candidate Against COVID-19 and Commence Pivotal Phase 2/3 Global Study*. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-choose-lead-mrna-vaccine-candidate-0, 2020.
91. Hassett, K.J., et al., *Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines.* Mol Ther Nucleic Acids, 2019. 15: p. 1-11.
92. Wrapp, D., et al., *Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.* Science, 2020. 367(6483): p. 1260-1263.
93. Mulligan, M.J., *Phase 1/2 Study to Describe the Safety and Immunogenicity of a COVID-19 RNA Vaccine Candidate (BNT162b1) in Adults 18 to 55 Years of Age: Interim Report* medrxiv, 2020.
94. Kariko, K., et al., *Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA.* Immunity, 2005. 23(2): p. 165-75.
95. Di Liegro, C.M., G. Schiera, and I. Di Liegro, *Regulation of mRNA transport, localization and translation in the nervous system of mammals (Review).* Int J Mol Med, 2014. 33(4): p. 747-62.
96. Vargas, D.Y., et al., *Mechanism of mRNA transport in the nucleus.* Proc Natl Acad Sci U S A, 2005. 102(47): p. 17008-13.

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