Molecular Therapy
Editorial

# Pros and Cons of Adenovirus-Based SARS-CoV-2 Vaccines

Most of us might be surprised by the rudimentary scientific rationale prevalent in the field of vaccine research just 50 years ago. For over a century after Louis Pasteur's vaccine against rabies, approaches usually consisted of inactivating a virus, injecting it, and seeing if it protected the host. Unlike today, interactions between vaccinologists and immunologists to improve vaccine efficacy were marginal.

With the rise of molecular biology, vaccine designs became more nuanced and the use of viral vectors emerged. An example is the evolution and checkered history of vaccines based on adenoviruses (Ads). Live Ad types 4 (Ad4) and 7 (Ad7) have been used in North American military recruits since the 1950s to prevent severe respiratory illness.[1] Similarly, dogs in western countries are vaccinated with an attenuated canine Ad type 2 (CAV-2) to prevent infection of the more virulent CAV-1.

Many of the first replication-defective Ad "vectors" in the early 1980s were vaccines. The original Ad vaccine design was relatively simple: delete a region of the viral genome that the virus needs to propagate, provide these functions via transcomplementing cells (e.g., Frank Graham's 293 cells) so that one could grow the vaccine, and then insert into the virus genome an expression cassette encoding the targeted epitopes.

Fast forward to 2020. The SARS-CoV-2 pandemic may be headed toward historic proportions—although still far from the 1918 Spanish flu (50 million deaths) and AIDS (35 million deaths)—inflicting havoc on families, communities, and economies and overwhelming health care facilities. Clearly, we need a vaccine. Are Ad-based vaccines targeting the SARS-CoV-2 spike and capsid proteins our best bet? After almost 70 years of working with Ads, their biochemical properties are well characterized: Ads are simple to make (in ~2 weeks a graduate student could generate enough of a novel Ad vaccine to treat a thousand mice and dozens of monkeys), easy to purify to high titer, genetically stable, easily stockpiled, relatively inexpensive, and can be delivered via aerosol, oral, intradermal, and intramuscular routes. The aerosol route is particularly relevant when targeting a respiratory virus because inducing protective immune responses that home to the tissue where infections will occur is strategically important. It is also worth noting that Ad-based vaccines tend to induce B cell and T cell responses.

Hundreds of millions of euros, dollars, and yen have been invested in advancing Ad-based vaccines. These advances include production and purification methods, genetic incorporation of epitopes into the capsid so that mononuclear phagocytes present these antigens via major histocompatibility complex (MHC) class I and II pathways, cloaking the capsid with polymers/shields or using Ad types with a lower level of seroprevalence to prevent neutralization by antibodies (NAbs) to common types found in many individuals, retargeting the vector to professional antigen-presenting cells, using helper-dependent vectors (so that the vector-infected cell only expresses the target epitopes and not Ad antigens), and single-cycle replication of vaccines to produce massive amounts of antigens. Each tweak, alone or in combination with others, has improved vaccine efficacy in preclinical trials.

As SARS-CoV-2 became a pandemic, it is astonishing that, in the case of the Ad-based vaccine frontrunners, little has changed from the basic design of 40 years ago. Some used the well-trodden path of an Ad5-based vaccine, while others switched to human (e.g., Ad26) or simian (monkey and gorilla) Ads that have low seroprevalence in Europe and North America (but not necessarily in Africa or Asia).[2] Conceptually, Ad type switching to avoid NAbs is at least 30 years old. The advent of simian Ad vaccines was not developed following a rigorous testing of all of the >200 different Ad types but was most likely the result of intellectual property issues and the ability to produce simian Ads in good manufacturing practice (GMP)-compliant cells. One presumes that subsequent rounds of Ad-based coronavirus disease 2019 (COVID-19) vaccine candidates will be more sophisticated.

Should we go "all in" on an Ad-based vaccine against SARS-CoV-2? The first issue is safety. There are few drugs or biologicals that do not have side effects or cause adverse reactions. Weighing the advantages versus disadvantages during the current pandemic can be idiosyncratic, and the strength of the reasoning varies by population, culture, religious beliefs, and bizarrely (for those of us outside the USA) even political affiliation. Current criteria limit the window to identify adverse reactions to 2 months. In addition to swelling and pain at the injection site, common to some vaccines, Ad-based vaccine adverse effects include fever, pneumonia, diarrhea, transient neutropenia and lymphopenia, fatigue, labored breathing, headaches, liver damage, and fasting hyperglycaemia. Rare but grave adverse reactions include neuropathies such as Bell's palsy, Guillain-Barré syndrome, gait disturbance, and transverse myelitis, an inflammatory condition in the spinal cord.

The origin of these effects likely lies in pre-existing anti-Ad immunity. Most adults have been infected by multiple Ad types and have persistent Ad infections. Together, this promotes long-lived anti-Ad B cell and T cell responses, include regulatory T cells ($T_{regs}$) that can dampen T cell responses. When injected with a bolus of Ad antigens (the vaccine), the response includes re-activation of anti-Ad effector memory T cells ($T_{EMs}$), which return via homing receptors to the mucosal environments—where most Ad infections occur—and

increased production of antibodies. Why are mucosal homing anti-Ad $T_{EMs}$ important? Some pathogens, like HIV, infect activated T cells in the mucosa.[3] If you live in an environment where HIV acquisition is a risk, then the last thing you want to do is provide HIV with an easy target. Increased HIV spread will fall below the radar of vaccine safety criteria because it is typically not included as an endpoint measurement.

An ideal vaccine should provide rapid, multifaceted, long-term protection. Few can argue with the preclinical data that demonstrate that Ad-based vaccines generate rapid, antigen-targeted immune response in mice, rabbits, hamsters, and monkeys. Assays in mice raised in a pathogen-free environment have suggested that Ad-based vaccines are fabulously efficient at generating rapid B cell and T cell responses. While most readily admit that data in mice are suggestive and vaccines need to be trialed in hosts (i.e., monkeys) with a complex immunological history (many monkeys will host their own Ad types) and diverse genetic backgrounds, clinical trials are being launched with mouse data. However, the number of monkeys used in preclinical studies is too small to provide robust safety evaluation. Moreover, I cannot recall preclinical vaccine studies that included a challenge step 12 months post-vaccination. Yet, such data would be critical because SARS-CoV-2 will not disappear within the foreseeable future. Hence, efficacy is not about whether Ad-based vaccines can prevent infections for 2 months but whether they will protect us for 12–24 months (although this caveat is not limited to Ad-based vaccines).

Most data suggest that the immune response to coronavirus is transient (<6 months).[4] With regard to COVID-19, the more severe the clinical complications, the greater the immune response against SAR-CoV-2, as is the case for most viral infections. Therefore, asymptomatic/mild COVID-19 would induce a lower immune response that would disappear within weeks. Fold this onto the kinetics and efficacy of an Ad-based vaccine, and the law of parsimony suggests that we need long-term antigen expression in a favorable environment to generate long-term protection. Yet, in ~70% of us our anti-Ad $T_{EMs}$ will target vaccine-infected cells. Is it possible that Ad-specific $T_{regs}$ could prevent lysis of vaccine-infected cells and allow long-term expression of SARS-CoV-2 antigens? This would be a welcome surprise. Follow-up studies from HIV and Ebola Ad-based vaccines suggest that a 4-dose vaccine regimen can induce immune hallmarks of protection for up 2 years. But the idea of 4 doses/person for billions of people during the first round of vaccinations—perhaps followed by booster shots every year—is difficult to imagine. While at least one phase 1 trial suggested that a 1-shot Ad-based vaccine could be sufficient, it is probable that this will be transient protection in most adults and particularly ephemeral protection in seniors.

Although Ad-based vaccines could be used alone by including type switching, the inclusion of other vaccine platforms (other viral vectors, proteins/peptides, nucleic acid-mediated expression, virus-like particles, inactivated or attenuated coronaviruses, etc.) may reduce the limitations of each platform and increase the breadth of the immune response. If phase 3 trials demonstrate that Ad-based vaccines are safe and efficient, I will be in line with my shirt sleeve rolled up. Looking forward, another key question is whether we must look beyond the 19th century mentality with the aim of generating vaccines that prevent COVID-19 as opposed to SARS-CoV-2 spread, but that is a subject for a future editorial.

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Eric J. Kremer[1]

[1]Institut de Génétique Moléculaire de Montpellier, University of Montpellier - CNRS, Montpellier, France

**Correspondence:** Eric J. Kremer, Institut de Génétique Moléculaire de Montpellier, University of Montpellier - CNRS, Montpellier, France.
**E-mail:** eric.kremer@igmm.cnrs.fr

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