Viruses, Vaccines and the Race for a Covid-19 Vaccine: A Tutorial

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Abstract

This tutorial is organized into three major sections—viruses, vaccines and the race for a Covid-19 vaccine. The goal is to provide enough background on viruses, history of vaccines, and the science of vaccinology founded on the principles of immunity. The hope is that this will enable us to understand the challenges, methods and prospects for developing a safe and effective vaccine against SARS-CoV-2. Many important viruses such as smallpox, HIV, HCV and SARS-CoV-2 which is responsible for causing the Coronavirus disease 2019 (Covid-19) are presented in detail, which is then followed by a description of different vaccine development methods and strategies. The tutorial then discusses different candidate SARS-CoV-2 vaccines and provides specific details of many of the prospective vaccines on the leader-board which are undergoing clinical trials. The tutorial concludes with a realistic projection for a safe and effective vaccine against SARS-CoV-2 based on the historical scientific record.

Historical introduction

We are currently in the midst of an evolving pandemic which is posing the greatest challenge to our health and well-being that humans have encountered in over a hundred years. The virus responsible for the pandemic, SARS-CoV-2, has infected more than 26 million people resulting in more than 875,000 deaths worldwide. The US currently has the highest number of infections of any country, with more than six million cases, and over 191,000 fatalities. Brazil comes in second with more than 4 million cases and is approaching 125,000 deaths. And India is third with close to 4 million infections and 69,000 fatalities.

To develop a critical understanding of the coronavirus disease 2019 (Covid-19) and its causative agent SARS-CoV-2, and to study the evolution of the pandemic over time in different parts of the world, it is necessary to examine the challenges various viral infections posed over the centuries, and the role of vaccines in containing them.

Smallpox became a scourge in the Indian subcontinent more than two millennia ago, and gradually spread to other parts of the world. Though a vaccine was developed for the disease by Edward Jenner in the later part of the 18th century, the concept of virus evolved during the last decades of the 19th century and crystallized only in the early years of the 20th century. As nicely put by William Summers, viruses are a conceptualization of scientists. The “virus” described by Jenner in 1778 during smallpox vaccination, and the view provided by Pasteur at the time of his rabies vaccine in 1885, and the “virus” concept provided by Stanley in 1935 when he crystallized the poliovirus are naturally different [1]. The first human virus to be discovered was the yellow fever virus in 1901 and the first to be visualized was the tobacco mosaic virus in 1939 using an electron microscope [2]. Throughout the 19th and first half of the 20th century all the vaccines were developed using viruses grown in animals and chicken egg embryos. This was the methodology for the development of vaccines for smallpox, rabies, yellow fever and influenza. However,

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the successful growth of the poliovirus in cell cultures led to the development of the polio vaccine in the early fifties of the 20\textsuperscript{th} century using a cell culture approach [2].

Emerging viruses cause new human infections that have never been encountered earlier, and reemerging viruses cause opportunistic infections after lying quiescent for many years, or even decades. Most emerging viruses are zoonotic, jumping to man from wildlife reservoirs, or through an intermediate domestic animal host. HIV, avian influenza, Nipah, SARS-CoV, MERS-CoV, Ebola, Lassa, Zika and the more recent SARS-CoV-2 are prominent emerging viruses. The Ebola virus reemerged in the nineties after the first outbreak of the seventies of the last century.

Viral evolution plays a significant role in the agent’s survival. By their mechanisms of mutation and recombination, the survival of many viruses is ensured, as variants arise which can evade the host immune responses. A classic case in point is the Influenza A virus which has not been eliminated even in the presence of effective vaccines and antiviral drugs such as amantadine and oseltamivir [2].

Viruses have also been found to be the causative agent for many tumors. In 1911 Peyton Rous first demonstrated the transmission of sarcoma in chickens through cell-free filtrates. Since then, over the course of a century, many viruses including human papillomaviruses (HPV), Epstein-Barr virus, Kaposi sarcoma herpesvirus, human T-cell leukemia virus, hepatitis B virus (HBV) and hepatitis C virus have been implicated in various types of cancers, together causing 15\% of human cancer deaths [3]. Vaccines have also been developed to prevent infection against some of these viruses, for example, HPV and HBV [4].

Working with the fowl cholera bacterium, Louis Pasteur developed a method of growing and weakening the organism in culture. He discovered that these weakened or attenuated bacteria caused a mild form of the disease and protected the chickens when exposed to the virulent form. Pasteur gave the name vaccine to this attenuated strain based on the Latin vacca which means cow. He was honoring Dr. Jenner and his work using cowpox inoculation to prevent smallpox [5].

The rest of the tutorial is organized as follows. We first consider a representative selection of the important viruses including SARS-CoV-2 in additional detail. It is followed by a discussion of the science of vaccinology incorporating the basic concepts of the various types of immunity, and the different stages of the vaccine development process to ascertain safety and efficacy. We then proceed to describe the prospective vaccines under various stages of development with a focus on those undergoing Phase III clinical trials. Finally, we provide a probabilistic estimate for a successful vaccine development outcome over the next two years.

Viruses

Smallpox virus

The description of this virus is summarized from the chapter on poxviruses provided in [6]. The smallpox virus, also referred to as the variola virus (VARV), belongs to the family of poxviridae that are DNA viruses. The cowpox virus (CPXV), the monkeypox virus (MXPV) and the vaccinia virus (VACV) also are members of the pox family of viruses. CPXV was originally used by Jenner for vaccination against smallpox, and it was subsequently replaced by VACV, and smallpox was finally eradicated from the planet in 1977. The common clinical presentation of smallpox is fever followed by skin rashes, which start as macules and evolve into papules, vesicles, and pustules over the course of a week. Subsequently, the pustules become umbilicated and dry up with the formation of scabs.
After the eradication of smallpox in 1977, WHO recommended ceasing vaccination in all countries of the world in 1980. Due to considerations of bioterrorism, US has recently developed a new smallpox vaccine which got FDA approval in 2007. US and Russia have large stockpiles of smallpox vaccines and are possibly immunizing their military personnel to protect against smallpox.

Varicella-Zoster virus
The description in this section is based on the chapter on the virus in [7]. Varicella-zoster virus (VZV) causes two completely different kinds of diseases, a primary infection called varicella or chickenpox, and a secondary belated manifestation called zoster or shingles. Zoster is manifested by reactivation of latent VZV due to declining cellular immunity over the years and is not transmitted directly from patients with chickenpox. Second attacks of chickenpox, though rare, can occur and are more common in immunocompromised individuals. More than 4 million cases of chickenpox used to occur annually in the US before vaccination was introduced in 1995.

Varicella presents with fever and a generalized itchy rash which lasts for 4-5 days. The rash is distributed more on the trunk and head. A few hundred skin lesions appear, many of which are vesicular. The vesicles heal without scarring.

Zoster manifests as a localized skin lesion spread over 1-3 dermatomal segments. The rash distribution is unilateral.

Active immunization for varicella is done with a live attenuated vaccine. It was originally developed in Japan in the seventies, but was approved in the US only in 1995. It can be given to healthy children and adults. Zoster can be prevented by immunizing elderly persons with a high dose of varicella vaccine (x14), and is marketed as Zostavax in the United States at this dosage.

Antiviral drugs Acyclovir (ACV) and Famciclovir are effective in reducing the severity of the diseases in varicella and zoster.

The structural characteristics of select viruses are summarized in Table 1. The epidemiological features, clinical manifestations resulting from these viral infections, and additional characteristics of these viruses are included in Table 2.

Table 1: Structural characteristics of select viruses. HIV: Human immunodeficiency virus, HCV: Hepatitis C virus, kbp: kilo base-pairs, kb: kilo base

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Molecular type</th>
<th>Structural Dimensions</th>
<th>Nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox virus</td>
<td>Double-stranded DNA</td>
<td>200-400 nm</td>
<td>130-375 kbp</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>Double-stranded DNA</td>
<td>80-120 nm</td>
<td>~125 kbp</td>
</tr>
<tr>
<td>HIV</td>
<td>RNA</td>
<td>100-150 nm</td>
<td>~10 kb</td>
</tr>
<tr>
<td>HCV</td>
<td>RNA</td>
<td>55-65 nm</td>
<td>~10 kb</td>
</tr>
</tbody>
</table>
Table 2: Epidemiological features and other relevant characteristics of select viruses. VZV: Varicella zoster virus, HIV: Human immunodeficiency virus, HCV: Hepatitis C virus.

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Mode of Transmission</th>
<th>Incubation period</th>
<th>Clinical condition</th>
<th>Vaccine yes/no</th>
<th>Eradication yes/no</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox virus</td>
<td>Airborne Droplets</td>
<td>10-14 days</td>
<td>Fever and rash</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Varicella-zoster virus (Varicella)</td>
<td>Airborne</td>
<td>10-23 days</td>
<td>Fever and rash</td>
<td>Yes</td>
<td>No</td>
<td>Acyclovir (ACV)</td>
</tr>
<tr>
<td>VZV (Zoster)</td>
<td>Reactivation of latent VZV from ganglia</td>
<td>Variable (years)</td>
<td>Localized skin rash in dermatome(s)</td>
<td>Yes</td>
<td>No</td>
<td>ACV Famciclovir</td>
</tr>
<tr>
<td>HIV</td>
<td>Sexual contact Blood transfusion</td>
<td>Years</td>
<td>Opportunistic infections</td>
<td>No</td>
<td>No</td>
<td>Anti-retroviral combination therapy</td>
</tr>
<tr>
<td>HCV</td>
<td>Injection drug use Contaminated needles and medical instruments</td>
<td>2 weeks – 2 months</td>
<td>Liver cirrhosis Liver cancer</td>
<td>No</td>
<td>No</td>
<td>Direct antiviral drugs are available</td>
</tr>
</tbody>
</table>

Human immunodeficiency virus
The summary of the human immunodeficiency virus (HIV) that follows is based on the description given in [8]. Worldwide, there were 38 million people living with HIV in 2018, with 1.7 million new infections for the year and there were 800,000 HIV related deaths in the same year (http://www.who.int/hiv/data/en/).
HIV is prevalent all over the world and is marked by a progressive weakening of the immune system, rendering the infected individual susceptible to various infections, and is typically fatal if left untreated.
Africa bears a disproportionate burden with more than two-thirds of the caseload. HIV is the causative agent of the acquired immunodeficiency syndrome (AIDS) which was recognized as a clinical entity in 1981. There are two types of viral strains, HIV-1 and HIV-2, but HIV-1 is the predominant one. Both are enveloped RNA viruses and belong to the family Retroviridae.

The virus replicates by attaching to a target cell using envelope glycoprotein gp120 which binds to CD4 cell receptor and the replication cycle is completed in 24 hours. See Figure 1 for details of the HIV-Virion structure. The primary cell targets of HIV are the CD4 T-lymphocytes and macrophages. The virus is inactivated by UV light and heat.

Epidemiology of HIV
The two strains HIV-1 and HIV-2 are separated by geography and hence affect different populations. HIV-2 is largely confined to West Africa while HIV-1 has spread globally. Rarely, mixed infections can also occur.
Transmission occurs through contact with infected body fluids—blood, semen, vaginal secretions and breast milk. The most common transmission setting is sexual contact, but infection can also occur by the transfusion of contaminated blood products. Transmission to the child from mother can occur during pregnancy, delivery, or breastfeeding. The average incubation period, measured as the asymptomatic period from the time of infection to the development of clinical manifestations of immunodeficiency, AIDS, is about 10 years. This occurs when the CD4 cell count falls from the normal range of 600-1000 cells/µl to <200 cells/µl.

Clinical manifestations
Loss of CD4 cells leads to opportunistic infections and malignancies, gradually affecting various systems of the body.

Prevention
The most effective preventive measure is adoption of safe-sex practices. Mother to child transmission can be prevented by perinatal drug interventions to the mother and child. Heating the breast milk before feeding it to the child kills the virus in the milk and makes it safe.

Vaccine status
Currently, there is no effective vaccine for HIV. The following are some significant challenges to the development of an effective vaccine: (1) Antigenic diversity of HIV proteins (2) Genetic diversity of the virus and high mutation rates (3) Viral genome integrates readily to the host cell chromosome and (4) The major target of the virus is the immune system itself.

Treatment
Effective combination therapy in one pill daily format is available which can be taken for decades. The goal is to achieve and sustain prolonged suppression of viral replication. There is currently no cure because of the persistence of latent viral reservoir even after decades of effective anti-retroviral treatment. Hence treatment needs to be continued lifelong.
Hepatitis C virus

The following description of the virus is adapted from [9] and [10]. Hepatitis C virus (HCV) is a single stranded RNA virus. The virus was identified in 1989. See Figures 2 and 3 for the structural and genomic characteristics of HCV.

**Epidemiology**

HCV infection is present in 3% of the world’s population. In the developed world injection drug use is the main mode of transmission while in developing countries it is mostly iatrogenic, via contaminated medical instruments, and unsafe injection practices. It is also sexually transmitted.

**Clinical manifestations**

Following infection with HCV, patients typically develop a mild form of acute hepatitis with nausea, loss of appetite, jaundice and elevated liver enzymes. A third to half of these cases resolve and the rest develop a chronic form of hepatitis, Chronic Hepatitis C (CHC). This can progress over time to liver cirrhosis. A subset of these patients goes on to develop hepatocellular carcinoma.

**Vaccine status**

Currently, there is no effective vaccine for HCV. Some of the well-recognized challenges to successful vaccine development are (1) Worldwide genetic diversity of HCV, (2) HCV’s ability to evade antibody responses and (3) the capability of HCV to suppress cell-mediated immunity.

**Treatment**

Directly acting combination antiviral therapy is currently available.
SARS-CoV-2

The coronavirus SARS-CoV-2 is the causative agent for coronavirus disease 2019 (Covid-19). SARS-CoV-2 is a respiratory tract virus which belongs to the viral family coronaviridae, also referred to as the coronavirus family [11]. Other prominent members of the respiratory tract group of viruses are the rhinovirus, the respiratory syncytial virus (RSV) and the influenza and parainfluenza viruses. The coronaviruses are single-stranded RNA viruses, containing an RNA inner core with an outer oily lipid envelope from which crown-like spikes of proteins project outwards. These characteristic crown-like projections on their surface give the virions the appearance of a solar corona in electron micrographs and hence the nomenclature “corona”. See Figure 4. The corona viruses are heat sensitive and are susceptible to lipid solvents such as acetone, ether, and vinegar (which contains acetic acid). The lipid envelope of the virus also breaks apart on contact with soap.

Figure 4: Eckert and Higgins illustration of SARS-CoV-2, Centers for Disease Control and Prevention, USA

The viral sequence of SARS-CoV-2 identified by Zhu et al. contains 29,892 nucleotides [11] and the viral genome reported by Wu et al. contains 29903 nucleotides [12]. See Figure 5 for additional genetic details. Phylogenetic analysis revealed the close relationship to SARS-like coronaviruses previously found in bats.

Figure 5: SARS-CoV-2 Genome

The viral sequence of SARS-CoV-2 identified by Zhu et al. contains 29,892 nucleotides [11] and the viral genome reported by Wu et al. contains 29903 nucleotides [12]. See Figure 5 for additional genetic details. Phylogenetic analysis revealed the close relationship to SARS-like coronaviruses previously found in bats.
in China. The pangolin is also likely to be an intermediate host and a natural reservoir of SARS-CoV-2-like coronaviruses [13].

Laboratory diagnosis
The lab diagnosis of SARS-CoV-2 infection is performed by real-time reverse transcription polymerase chain reaction (RT-PCR) assay for a genetic sequence matching the genome of SARS-CoV-2. This is accomplished by SARS-CoV-2 specific primers and probes. SARS-CoV-2 is a respiratory virus which is shed in respiratory droplets. A swab taken from the deep nasopharynx is used to isolate the virus from an infected person. Figure 6 provides additional details of the SARS-CoV-2 RT-PCR test.

Epidemiology of SARS-CoV-2
Covid-19 is an evolving pandemic and currently has infected more than 26 million people and caused more than 875,000 deaths worldwide. The virus is highly contagious with a mean incubation period of 5.5 days and a range of 2 days to 12 days.

Clinical features
SARS-CoV-2 is a respiratory virus which predominantly affects the respiratory system causing bilateral pneumonia. It is also known to cause cardiovascular, neurological, gastrointestinal and skin manifestations in some patients. See [14] for detailed clinical features of Covid-19.

Vaccine status
Currently, there is no effective vaccine for preventing infection with SARS-CoV-2. Trials of candidate vaccines are ongoing.
Treatment
No specific anti-viral treatment is currently available broadly for Covid-19.

Vaccines and vaccinology
The science of vaccinology is founded on the basic principles of immunology which we discuss below.

Innate and adaptive immunity
The human body fights infections using two basic immune mechanisms called innate and adaptive immunity. Innate immunity is the body’s first line of defense and is activated immediately within minutes to a few hours of the entry of pathogens. Macrophages and neutrophils are recruited to quickly engulf and destroy extracellular pathogens by phagocytosis. Pathogen recognition is achieved in an antigen-specific manner using related molecular structures called pathogen-associated molecular patterns (PAMPs). PAMPs are broad patterns made up of essential polysaccharides and polynucleotides found in various pathogens but these are absent in the host receptors. In innate immunity there is no memory trigger of past exposures. “Natural antibodies” which are non-specific immunoglobulins also play a role in innate immune mechanisms.

Adaptive immunity is a tailored and specific response to pathogen entry and the body mounts an adaptive immune response over a period of a few days. The pathogen is recognized precisely by receptors which are generated by a random process of differentiation of B and T lymphocytes. Clones of highly specific T lymphocytes that can discriminate between even very small differences in the molecular structure of pathogen epitopes are generated and recruited for the adaptive immune response. The pathogen epitopes are made of polypeptides or polysaccharides and represent the uniqueness of a pathogen. A primary adaptive response takes about a week but the memory of the event is retained and a secondary response typically has a shorter lag phase of three or four days. Moreover, in the primary response low-affinity IgM antibodies predominate while high-affinity IgG antibodies dominate the secondary response. See Figure 7 for a depiction of the primary and secondary immune response curves. Figure 8 shows the interaction of innate and adaptive immune systems. The interested reader is referred to [5, 15] for more details.
Herd immunity

In vaccinology, the concept of herd immunity plays a significant role. When a community attains herd immunity it can be expected to be protected from the onslaught of a specific pathogenic infection. One way to obtain this type of immunity is via the natural course of disease transmission when a significant proportion (60-95%) of individuals in a community is exposed and subsequently gets infected by the pathogen. However, this can impose a high burden of mortality and morbidity on the population. A desirable approach to attain herd immunity is through vaccination or immunization. However, the proportion of a population that must be immunized with a vaccine for a specific infectious disease is dependent on the transmissibility of the pathogen in question. For example, measles is a highly contagious disease, and for effective protection, a 95% coverage is needed. This is to ensure that the entire population is shielded from the pathogen. Herd immunity confers a direct protective effect on the immunized, but it also provides indirect protection for the non-immunized individuals by disabling the transmission chain as shown in Figure 9.

History of vaccines

From a functional perspective there are two predominant categories of vaccines, prophylactic and therapeutic. Prophylactic vaccines are typically administered as part of a preventive strategy before a person is exposed to a pathogen or develops the disease. Therapeutic vaccines are administered as a treatment strategy for an existing disease.
Generally, Edward Jenner is credited with developing an approach to Smallpox vaccination in 1796 using previous observations that milkmaids who developed cowpox lesions were protected from Smallpox. However, Benjamin Jesty, a farmer, had also become aware of the protection from Smallpox that an earlier cowpox infection provided. Based on this knowledge Jesty inoculated his wife and two sons with fluid from a cowpox lesion on one of his cows in 1774 [16]. Likewise, the credit for sheep Anthrax vaccine goes to Louis Pasteur who made a public demonstration of his anthrax vaccination methodology in 1881 by inoculating 25 sheep, with another 25 sheep retained as controls. When challenged with anthrax spores 24/25 vaccinated sheep survived compared with only 2/25 survivors in the control group. However, Pasteur did not give credit to Henri Toussaint who developed the method to inactivate anthrax bacteria using phenol [16]. Later, Pasteur developed a killed vaccine for rabies from air-dried spinal cords of infected rabbits. He successfully vaccinated a 9-year-old boy, Joseph Meister, bitten by a rabid dog in 1885 and Meister survived. This was the first therapeutic vaccination, as Meister was vaccinated after exposure to rabies.

Starting with serendipity and observation in the late 1700s, vaccine methodologies evolved into empirical approaches involving isolation, inactivation, and also the use of killed microbes, in the late nineteenth and first half of the twentieth centuries. For diphtheria and tetanus, toxins were identified as the cause of the disease, and antiserum made against toxins in horses was used in vaccination. During the period of 1880 to 1950, vaccines were developed successfully to tackle rabies, diphtheria, tetanus and polio. The BCG vaccine for tuberculosis was also developed during this period.

The second half of the twentieth century saw two new successful vaccine development approaches—recombinant DNA technology, and glycoconjugation or polysaccharide-protein conjugation. By 1980 the recombinant method yielded two effective vaccines, for hepatitis B, and the human papillomavirus (HPV). And by 1990, the polysaccharide-protein conjugation method enabled the development of the meningococcal ACWY (MenACWY), haemophilus influenzae type b (Hib), pneumonia and staphylococcus aureus vaccines. These newer vaccines are also referred to as subunit vaccines, as they contain only parts of the pathogen, but are capable of generating an adequate immune response following vaccination. In the first decade of the twenty-first century, genome-derived reverse vaccinology approaches using
bioinformatics methods were employed to develop the meningococcal B (MenB) vaccine. See Figure 10 for an illustration of the vaccination milestones.

![Figure 10: Vaccination milestones: Serendipity to empirical approaches to epitope prediction and reverse vaccinology](image)

**Public health impact of vaccines**

Twentieth century saw the development of new and effective vaccines for many infectious diseases resulting in a dramatic reduction in mortality and morbidity from the onslaught of these diseases. Just in the twentieth century, smallpox killed more than 500 million people before the worldwide eradication strategy of the disease succeeded and smallpox was eliminated as a disease in 1980. Though effective vaccines have been developed for Influenza, about 30,000 people on average succumb to the complications resulting from Influenza in the US each year. This translates to approximately half a million deaths globally every year from the disease. Measles causes more than 100,000 deaths worldwide in spite of the availability of an effective vaccine from the 1960s. The reasons for the failure to control outbreaks of diseases in many countries of the world even when effective vaccines are available can be traced to economic disparities, widespread ignorance among the populace, lack of political will in the countries’ leadership, and inadequate allocation of resources for public health. However, a substantial increase in the life expectancy of children, and by extension, in the whole populace was observed globally in the second half of the twentieth century due to the impact of vaccination and immunization campaigns led by WHO and the respective public health authorities of various countries.

The dramatic reduction in mortality and morbidity of various infectious diseases observed in the last century in the US clearly illustrates the significant impact of vaccines in the prevention and control of various infectious diseases. By the end of the century, the percentage reduction in morbidity for smallpox, diphtheria, pertussis, tetanus, paralytic polio, measles, mumps and rubella varied between 99.3 percent to 100 percent. See Table 3 for additional details. Paralytic polio is on the verge of global eradication, having been eliminated from the developed world and most developing countries. However, there are isolated reports of paralytic polio during the last nineteen months (January, 2019 to July, 2020) in five countries of Asia (Afghanistan, China, Myanmar, Pakistan, Philippines) and fourteen countries of Africa [17]. Among these countries only Afghanistan and Pakistan have documented paralytic polio due to wild-type poliovirus. Paralytic polio cases in the other countries are oral polio vaccine associated paralytic poliomyelitis resulting from circulating vaccine-derived poliovirus. However, when transmission of wild-
type polio virus ends, eradication of polio would be achieved and oral polio vaccination could be discontinued.

Table 3: Annual morbidity due to common vaccine-preventable diseases recommended for universal use in US children before 1990

<table>
<thead>
<tr>
<th>Disease</th>
<th>20th Century Morbidity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>1998 Morbidity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Percent Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smallpox</td>
<td>48,164</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>175,885</td>
<td>1</td>
<td>99.99</td>
</tr>
<tr>
<td>Pertussis</td>
<td>147,271</td>
<td>6279</td>
<td>95.7</td>
</tr>
<tr>
<td>Tetanus</td>
<td>1314</td>
<td>34</td>
<td>97.4</td>
</tr>
<tr>
<td>Polio (paralytic)</td>
<td>16,316</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Measles</td>
<td>503,282</td>
<td>89</td>
<td>99.99</td>
</tr>
<tr>
<td>Mumps</td>
<td>152,209</td>
<td>606</td>
<td>99.6</td>
</tr>
<tr>
<td>Rubella</td>
<td>47,745</td>
<td>345</td>
<td>99.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average annual number of cases before universal vaccine use recommended (Smallpox (1900-04), Diphtheria (1920-22), Pertussis (1922-25), Tetanus (1922-26), Polio (1951-54), Measles (1958-62), Mumps (1968), Rubella (1966-68))

<sup>b</sup> Number of cases reported for each disease in 1998

[Adapted from MMWR, 1999, 48(12)]

Types of vaccines

As noted earlier, the field of vaccinology started with live attenuated and inactivated vaccines. During the last part of the twentieth century, and more so in this century, the focus has shifted to subunit vaccines particularly for tackling emergent viral diseases. These subunit vaccines typically consist of surface proteins that can induce a significant immune response and they could also be conjugated with polysaccharides. Subunit vaccines can also be engineered with DNA or RNA segments that encode for the immunogenic proteins. These vaccines are also referred to as recombinant vaccines. As the recombinant vaccines consist of specific immunogens, they elicit a better immune response than inactivated vaccines.
They are also considered safe when compared with live attenuated vaccines as there is no danger of virulence. The vaccine types are illustrated in Figure 11.

![Vaccine Types Diagram]

**Figure 11: Basic types of vaccines (modified from Introduction to molecular vaccinology, Giese, 2016)**

**Nucleic acid vaccines**

These vaccines use antigen-encoding plasmid DNA or RNA. In this case the vaccine does not possess the genome to make the microbe but just a subset of genes necessary to make the required immunogens needed for protective immunity. The DNA has to be delivered inside cells so that it can be transcribed first to messenger RNA (mRNA), and then translated into the relevant immunogenic proteins, making use of the cell machinery. The cells subsequently secrete the immunogens, or they get attached to the surface. If mRNA is injected, it is directly translated into proteins using the cellular machinery. Because mRNAs can be directly translated into proteins using the cellular ribosomal machinery, they have a superior protein (antigen) expression profile. Moreover, antigen-specific targeting is facilitated by the expression of specific antigens in antigen-presenting cells. And compared with DNA vaccines, mRNA-based vaccines are also not susceptible to mutagenesis [18]. The body’s own cells are responsible for manufacturing the vaccine using its own cellular machinery resulting in protective immunity. Both humoral and cell-mediated immune responses are generated by this method [19].

There are a few advantages for nucleic acid vaccines. These vaccines are cell-free. Since no live virus-handling is needed, a biosafety level 2 (BSL2) lab is not needed for vaccine development. Moreover, production can be speeded up as manufacturing is synthetic. The disadvantage is that mRNA is fragile and cold storage is needed to prevent degradation.

**Therapeutic vaccines**

Therapeutic vaccination involves the administration of a vaccine to manage or treat a preexisting disease condition. The general goal here is to target non-infectious diseases such as cancer, Parkinson's, Alzheimer’s, multiple sclerosis, arthritis, or conditions such as obesity, high blood pressure and drug addictions. The challenge is to critically understand the disease pathogenesis and pathology and come up with suitable targets for vaccination. Note that in the case of non-infectious diseases, we are dealing with host proteins as opposed to pathogen-derived proteins as targets, and this can cause problems with immune targeting. The recognition of specific conformational forms in the pathological processes
associated with the disease is a key step in the development of effective therapeutic vaccines [20, 21]. Identification of circulating cytotoxic T cells specific to tumor antigens indicates that induction of an immunogenic response to mutated tumor host proteins is feasible.

Therapeutic vaccines are broadly categorized into cancer vaccines and non-infectious non-cancer vaccines (NINC). Current cancer vaccines include personalized tumor immunotherapy, prostate cancer vaccine and the use of BCG vaccine for bladder cancer. NINC vaccines are an area of intense investigation, with vaccines for addiction, high blood pressure and obesity, currently in exploratory stages. See Figure 12 for more details.

![Figure 12: Non-infectious non-cancer (NINC) vaccines (modified from Introduction to molecular vaccinology, Giese, 2016)](image)

**Vaccines, immunity and age**

Age exerts a significant influence on the immune system. Generating immunological response by vaccination is easier in the young while immune protection is quite challenging in older people. Low efficacy of seasonal influenza vaccine in the elderly has been well-documented [22, 23]. Immune competence decreases with increasing age which is termed as immunosenescence. This can result from changes at multiple levels of the immune system over time, such as reduced lymphopoiesis in the bone marrow, and thymus atrophy. These in turn result in a lower output of native T lymphocytes. Likewise, T cell receptors and B cell receptors are also negatively impacted by aging. This implies that a conventional vaccine based on young adult responses may not be highly effective in older adults. Supplementation of vaccination with immunotherapy by an infusion of neutralizing monoclonal antibodies might be needed in the elderly for enhanced protection.

**Vaccine protection mechanisms**

It is critical to understand what vaccines can and cannot do. Vaccines do not prevent pathogen entry or transient pathogen growth. Stimulating the host immune response by activating innate and adaptive immune mechanisms, vaccines can block pathogens from establishing a firm foothold by inhibiting the growth, development and unchecked replication of these organisms. They can prevent the occurrence of
clinical manifestations of disease or reduce the severity and complications resulting from infections. The goal of vaccination is to induce adaptive immunity that is specific for vaccine antigens. This facilitates the development of persistent antibodies, and memory B-cell and T-cell responses. This cascade of events protects the vaccinated individual during later contact with the virulent pathogen. The vaccine components engage the innate immune mechanism to induce protective adaptive immune responses.

**Vaccine development process**

Creating an effective and safe vaccine involves the successful completion of five critical stages—exploratory, preclinical, clinical trials, FDA review/approval and finally manufacturing the required

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**Figure 13:** The five stages of the vaccine development process (Wikimedia)
number of doses. These stages are sequential and successful completion of a specific stage is necessary to move forward to the next stage. The exploratory stage incorporates in silico, medicinal chemistry and biological experimentation which leads to the preclinical stage, which typically involves testing for safety and efficacy on lab animals and possibility even primates. Successful completion of these two stages moves the process forward to the most important stage called the clinical trials stage which we will consider in some details. Figure 13 illustrates these five stages of the vaccine development process. The figure also shows the accelerated timeline with overlapping stages (stages 3, 4 and 5) for possible emergency use authorization in case of pandemics.

**Clinical trials**

A candidate vaccine advances to the clinical trials stage to evaluate the safety and efficacy of the vaccine in a rigorous and transparent manner. This task is accomplished by progressively larger and complex human trials which are conducted in three sequential phases—phase I, phase II and phase III. Safety of the candidate vaccine is assessed using healthy volunteers in phase I. In phase II, preliminary efficacy, dosage protocol, and additional safety determinations are made. The phase III clinical trial is the most critical, complex and challenging step of the vaccine development process. Typically, it is a large randomized, placebo-controlled and double-blinded clinical trial involving the enrollment of thousands or even tens of thousands of subjects based on strict inclusion and exclusion criteria. It is clearly an expensive and resource-intensive undertaking. Successful completion of this important phase is mandatory for regulatory review and approval, which then moves on to the manufacturing stage. See Figure 14 for additional details of the different phases of the clinical trials stage for a Covid-19 candidate vaccine.

*Figure 14: Major phases of a Covid-19 candidate vaccine clinical trial—phase I, phase II and phase III*
Vaccine development timeline

As the vaccine development process involves five sequential stages including a three-phase clinical trial stage, it takes many years to decades to develop a successful vaccine. For example, development of the Meningococcal B vaccine, including licensing, took almost fifteen years (see Figure 15). The shortest recorded time is for the live attenuated Jeryl Lynn mumps vaccine, developed by Maurice Hilleman, at Merck laboratories in a span of five years [24].

Figure 15: Reverse vaccinology framework and timeline for Meningococcal B vaccine development (modified from Human vaccines, Modjarrad and Koff, 2017)

Vaccine development failures

Even when the causative pathogen is isolated and sequenced, a safe and effective vaccine may not materialize in spite of persistent efforts. For example, there are no licensed effective vaccines currently in the market for HIV, hepatitis C and malaria. HIV mutates continuously in the human host which poses significant challenges for vaccine development. The hepatitis C virus shows enormous diversity, and animal models for testing potential vaccines are limited. Moreover, our understanding of protective immune responses against HCV are still evolving [25]. In general, developing vaccines against parasites is problematic because of larger genomes. The malarial parasite genome has more than 5,000 genes which provide hundreds of potential targets, and a plethora of vaccine candidates over the different stages of the parasitic life cycle. We need a better understanding of the mechanisms and key targets of immunity to develop a successful and durable vaccine for malaria [26].

Race for Covid-19 vaccine

Covid-19 has emerged as the biggest public health challenge mankind has faced over the last one hundred years, and the contagion continues to rage in many countries of the world. To combat the disease and bring the pandemic under control, various teams, spread over many countries, are in a race to develop a successful vaccine against SARS-CoV-2.
Vaccine candidate mechanisms
Apart from the more traditional approaches of using an attenuated and inactivated virus, most of the leading candidate vaccines use one of these three methods—mRNA coding a SARS-CoV-2 gene, recombinant SARS-CoV-2 surface protein, and viral vector packaging SARS-CoV-2 gene as the key mechanism to induce an effective anti-body response in the vaccinated host. This is illustrated in Figure 16.

Figure 16: Three key mechanisms used in SARS-CoV-2 candidate vaccines (Wikimedia)

Candidate vaccines
As of August 25, 2020, there are 31 candidate vaccines undergoing clinical evaluation, that is, they are in phase I, phase II or phase III trials. Another 139 candidate vaccines are in the pre-clinical stages of development. See Table 4 for additional details about these candidate vaccines.

Table 4: Number of prospective Covid-19 vaccines in clinical and pre-clinical phases. O ongoing, C completed, VLP: virus like protein

<table>
<thead>
<tr>
<th>Stage (Ongoing/Completed)</th>
<th>Number</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-replicating viral vector</td>
</tr>
<tr>
<td>Phase 3 (O)</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Phase (O/C)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Phase (O/C)</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>Pre-clinical</td>
<td>139</td>
<td>19</td>
</tr>
</tbody>
</table>
Let us look at a few of the leading candidate vaccines undergoing Phase 3 trials in some more detail.

**University of Oxford/AstraZenica (UOAZ) viral vector vaccine**
The UOAZ vaccine is undergoing two Phase III trials, one in the United States and the other in the United Kingdom. The US Phase III trial plans to recruit 30,000 adults eighteen years or older and study the safety and efficacy of the vaccine. See Figure 17 for additional details.

<table>
<thead>
<tr>
<th>Timeline</th>
<th>Study protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Start: August 2020</td>
<td>• Adults ≥18 years</td>
</tr>
<tr>
<td>• Completion: October 2022</td>
<td>• 2 IM doses of AZD1222 or placebo given 4 weeks apart</td>
</tr>
<tr>
<td>• Randomized double blind placebo-controlled</td>
<td>• Follow up visits at day 28, 80, 182 and 364</td>
</tr>
<tr>
<td>• Phase 3: 30,000 participants</td>
<td>• Test for Covid-19 if symptoms develop</td>
</tr>
<tr>
<td>• Vaccine type: non-replicating viral vector vaccine</td>
<td></td>
</tr>
</tbody>
</table>

**Outcome measures**
- **Primary**
  - PCR positive symptomatic Covid-19 over 12 months
  - Safety and tolerability of two IM doses compared to placebo
- **Secondary**
  - Prevention of SARS-COV-2 asymptomatic infection over 12 months
  - Periodic immunogenicity study

**Figure 17: University of Oxford/AstraZenica viral vector candidate vaccine**
The UK trial is combining Phases II and III with the stated goal of determining the efficacy, safety and immunogenicity of the candidate vaccine in adults (≥18 years) and children (2-11 years). The protocol calls for the assessment of humoral and cellular immunogenicity on days 0, 7, 14, 28, 42, 56, 182 and 364. The trial plans to enroll 60 children (2-11 years), 12,030 adults (18-64 years) and 240 older subjects (≥65 years). It will be a double-blind placebo-controlled trial.

**Moderna/NIAID mRNA vaccine**
This candidate vaccine is undergoing a Phase III trial to assess its safety, efficacy and immunogenicity in adults ≥18 years old. It will be a double-blind placebo-controlled trial. See Figure 18 for additional details of the clinical trial. Note that Moderna has completed the Phase I study successfully [27] but the Phase II study is ongoing. This mRNA vaccine encodes the S-2P antigen, consisting of the SARS-COV-2 glycoprotein with a transmembrane cover and constitutes a S1-S2 cleavage site.
Figure 18: Moderna/NIAID mRNA vaccine

**BioNTech/Pfizer mRNA vaccine**

This candidate vaccine is currently undergoing a randomized placebo-controlled multi-site Phase III trial to assess its efficacy and safety. The vaccine moved to Phase III after successfully completing a combined Phase I/II study. It is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine that encodes the trimerized SARS-CoV-2 spike glycoprotein receptor-binding domain. In the Phase I/II study using 45 healthy adult volunteers, the investigators observed 1.9x to 4.6x neutralizing antibody titer levels when compared with convalescent human serum [28]. See Figure 19 for additional details of the study.

Figure 19: BioNTech /Pfizer mRNA vaccine

**Sinovac vaccine**

The Sinovac candidate vaccine is currently undergoing a randomized placebo-controlled blinded trial in two sites—Brazil and Indonesia. It is an inactivated vaccine. The results of Phase I/II studies have not been published yet. The details of the Phase III study are shown in Figure 20.
The Novavax protein subunit vaccine is currently undergoing Phase I studies with an optional extension to Phase II. It is a double-blinded randomized controlled study which uses a recombinant spike protein nanoparticle configuration. Additional details of the vaccine and study protocol are shown in Figure 21.

Prospects, pitfalls and challenges of vaccine development during pandemic times

Based on the progress made so far, it is evident that vaccine development is proceeding at an accelerated pace compared to historical vaccine development efforts. There is the pressure of the pandemic, which is currently infecting more than a quarter million people daily, and causing more than six thousand fatalities on a daily basis worldwide [29]. The comparative daily counts for the US are more than 40 thousand new cases and more than one thousand deaths daily. India is also reporting more than 70 thousand cases, and fatalities in excess of one thousand in a single day. As we already noted, over the eight-month duration
of the pandemic, SARS-CoV-2 has infected more than 26 million people worldwide with total fatalities exceeding 875,000. These are the people who tested positive for the virus. Assuming the magnitude of infection to be ten-fold would still leave out more than 95 percent of the world’s population as potential targets of the virus. For the pandemic to resolve there need to be about 80 percent herd immunity in the population which could be acquired through infection or vaccination. Assuming current mortality rates, achieving herd immunity globally through spread of infection would result in additional fatalities upward of fifteen million. Hence developing and deploying a safe and effective vaccine globally is a much more desirable option. However, releasing a vaccine to sections of the general population, before completing a successful Phase III trial, and establishing the safety and efficacy of the candidate vaccine, is a very risky proposition.

While neutralizing antibodies have been demonstrated in persons recovering from an infection with SARS-CoV-2, it is not clear if the infection confers long-lasting immunity. The pandemic has been in existence for only eight months, and hence it is not feasible to infer the durability of immunity beyond a span of few months following a natural infection. Though published Phase I results have been encouraging, it is too early to make any long-term projections beyond a note of guarded optimism.

Vaccines for measles, mumps, rubella, polio, smallpox and influenza have a long history of safe use, and they were all developed with stringent regulatory requirements and scientific rigor. It is notable that the SARS-CoV-1 vaccine generated worrisome immune responses in ferrets and monkeys while mice were reportedly safe [30]. Anti-SARS-CoV-1 spike protein antibodies generated by a vaccinia vector, worsened lung injury in Chinese macaques after being administered a challenge dose of the virus, which was the result of infection and proinflammatory reprogramming of macrophages [31]. In general, vaccine-associated disease enhancement remains a distinct possibility. Lax regulation by FDA recently resulted in the marketing of many SARS-CoV-2 antibody tests with questionable sensitivity and specificity. That is a cautionary tale.

WHO has provided preferred target characteristics for a safe and effective SARS-CoV-2 vaccine [32]. Table 5 summarizes the desired properties for a Covid-19 vaccine.

Table 5: Preferred characteristics of a SARS-CoV-2 vaccine based on WHO guidelines

<table>
<thead>
<tr>
<th>Vaccine feature</th>
<th>What is preferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication</td>
<td>Active immunization to prevent Covid-19</td>
</tr>
<tr>
<td>Contraindication</td>
<td>None</td>
</tr>
<tr>
<td>Target population</td>
<td>All ages</td>
</tr>
<tr>
<td>Safety and reactogenicity</td>
<td>High benefit to risk profile</td>
</tr>
<tr>
<td>Efficacy</td>
<td>Minimum 70% efficacy</td>
</tr>
<tr>
<td></td>
<td>Onset of protection within 2 weeks of administration of vaccine</td>
</tr>
<tr>
<td>Dose regimen</td>
<td>Single dose plus annual booster if needed</td>
</tr>
<tr>
<td>Duration of protection</td>
<td>At least one year</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Non-parenteral preferred</td>
</tr>
<tr>
<td>Stability and storage</td>
<td>Shelf life of 6-12 months at room temperature</td>
</tr>
<tr>
<td>Accessibility</td>
<td>Capability to quickly scale-up production</td>
</tr>
</tbody>
</table>
Vaccine prospects

Based on the foregoing discussion we now attempt a realistic projection for a successful vaccine in the next couple of years. Figure 22 provides possible scenarios for July, 2021 and July, 2022.

![Figure 22: Vaccine outlook for July 2021 and July 2022](image)

Conclusion

Based on the foregoing discussion it is likely that we will have a safe and effective preventive vaccine for SARS-CoV2 within the next year or two. However, as our experience with some of the viruses demonstrates, a note of caution is in order. A safe and effective vaccine across all age-groups for SARS-CoV-2 cannot simply be guaranteed, but looks like a cautiously optimistic proposition.

References

17. Polio this week as of August, 2020 [http://polioeradication.org/polio-today/polio-now/this-week/]