ACCADEMIA NAZIONALE DEI LINCEI

COVID-19 vaccines: June 2020 report

Statement by the Lincei Committee on Covid-19

Responsibility for the information and views expressed in this document lies solely with the Covid-19 Committee

1. Introduction

The hope and hype that the media and public at large are placing on having as soon as possible a vaccine that protects against COVID-19 is the result of the

great triumphs that vaccines have had and are having in the control of infectious diseases. However, there is a long series of infectious diseases in which vaccines are only partially effective and we have a series of sensational vaccine defeats[1].

Indeed, each disease is an immunological problem in itself: even today, with all the data in our possession, it is difficult to predict what kind of vaccine can be truly effective. This difficulty is even greater for COVID-19, a young disease in which ongoing studies in laboratories worldwide are bringing new data. In addition, RNA viruses generally have a high mutation rate. This is one reason why it is difficult to develop effective vaccines to prevent diseases caused by RNA viruses. However, data on the SARS-CoV-2 RNA sequence suggest that this virus does not undergo frequent mutations, thanks to its efficient proofreading system.

In many cases, recovery from a viral disease rests on the combined action of antibodies in the biological fluids that neutralize viral particles and the killer activity of lymphocytes that track down and kill the body's cells infected with the virus. However, there are viral diseases whose healing depends mainly, if not exclusively, on the antibody response and others where the destructive action of the killer lymphocytes is fundamental. What is the case with COVID-19 is not yet defined. First data on T cell reactivity are only recently being published[2].

Often, healed patients display high titers of SARS-CoV-2 neutralizing antibodies. However, there are also healed patients with a low antibody titer[3]. Data on the role of secretory IgA and IgM are scarce. Furthermore, we cannot yet know how long the protection acquired by recovered patients lasts. Often the duration of protection after healing somewhat corresponds to the duration of the protection provided by the vaccine.

Despite the impressive amount of studies carried out since the virus was first characterized, there are still a large number of unknowns about this disease. And it is precisely these unknowns that fully justify the very different conceptual and technological strategies that are currently pursued in the preparation of vaccines against COVID-19. This diversification appeared essential precisely because, for many diseases, but mainly in the case of a new

disease as COVD-19, it is difficult to predict which type of immune response and therefore vaccine will be more effective [4].

Fig. 1, which was taken (with permission) by Lurie et al.[5], shows the difference between traditional vaccine development and development under the pressure of a rampant epidemic. Because of the pressure created by the pandemic, multiple activities are carried at financial risk to the developers, without knowing whether the vaccine candidate will be safe and effective, including very early manufacturing, scale-up and commercial scale, before the establishment of clinical proof of concept[5].

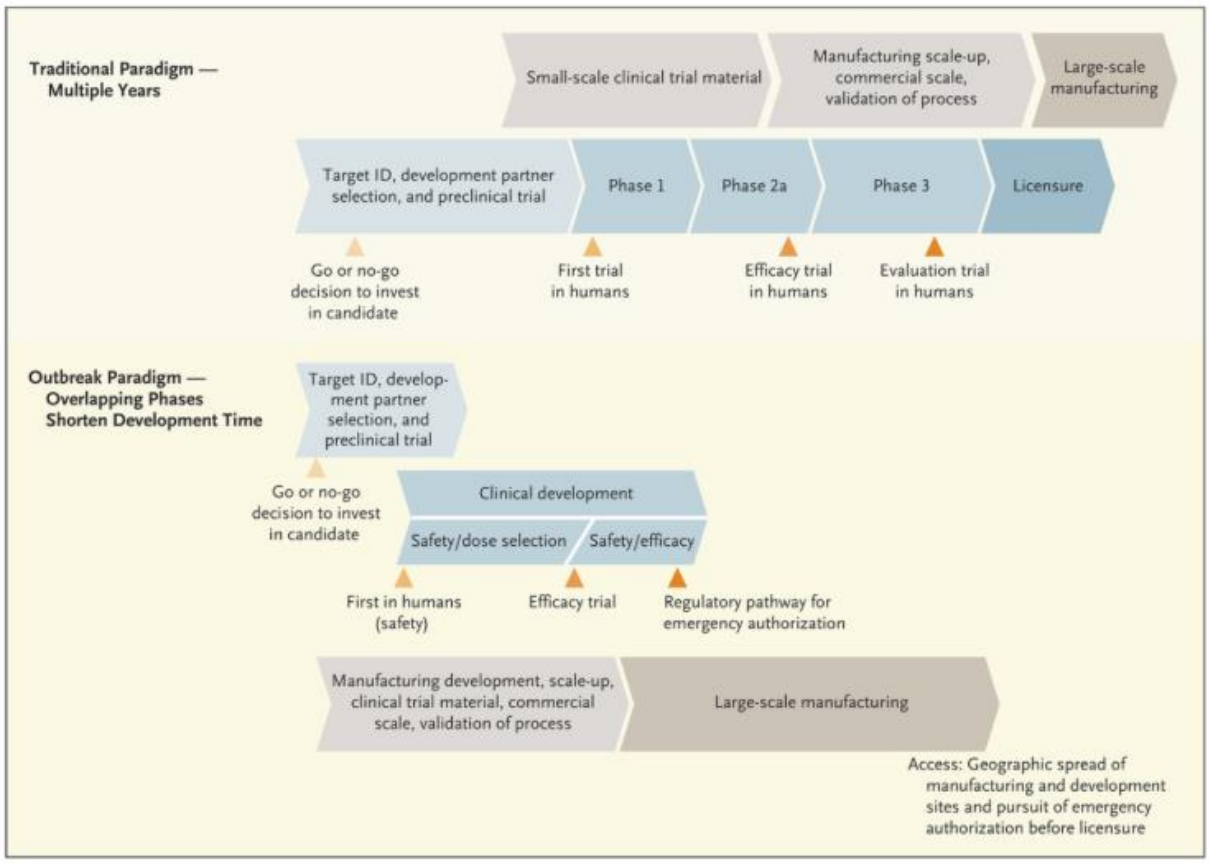

Fig. 1

2. Vaccine targets and technical strategies

As of June 2020, there are numerous anti SARS-CoV-2 vaccine candidate projects targeting the whole virion, molecules or fragments of molecules expressed on the virion surface. Among the numerous lists of vaccine projects

we refer to both The Scientist Journal list https://www.the-scientist.com/news-opinion/covid-19-vaccine-frontrunners-67382 and the WHO Draft landscape of COVID-19 candidate vaccines https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines.

Vaccine target antigens.

- Attenuated SARS-CoV-2 are the target antigens of two vaccine projects ongoing in India at The Serum Institute of India in collaboration with Codagenix, a New York private biotech, and at Indian Immunologicals Ltd in collaboration with the Griffith University, Australia.
- The inactivated whole SARS-CoV-2 virion is targeted by four independent projects ongoing in China at the Sinovac Biotech, the Wuhan Institute of Biological Products, Beijing Institute of Biological Products/Sinopharm, Institute of Medical Biology /Chinese Academy of Medical Sciences.
- A virus-like particle (VLP) mimicking SARS-CoV-2 produced in plants is the target antigen of the vaccine project of Medicago Inc, Canada. Nature inspired VLP COVID-19 vaccine is studies at Imophoron, Bristol, UK. A VLP scaffold to express SARS-CoV-2 antigens is studied at the SwissSaiba GmbH. Other VLP based vaccines are studied at Osivax, France, at the Doherty Inst., Australia, and at the German vaccine developer ARTES Biotechnology GmbH.
- By contrast, the whole Spike protein or its fragments, containing the S1 Receptor Binding Domain (RBD), are variously targeted by a great majority of the anti COVID-19 vaccine projects.
- Other SARS-CoV-2 proteins. A few other projects are targeting the nucleoprotein (N): MIGAL Galilee Research Inst, Israel, and DZIF German Center for infection Research, or the M protein (OncoGen, Romania) and various SARS-CoV-2 proteins besides the Spike protein.

Fig. 2 which was taken (with permission) by E. Callaway[6] shows the variuos strategies and technical platforms currently adopted for the development of the COVID-19 vaccines.

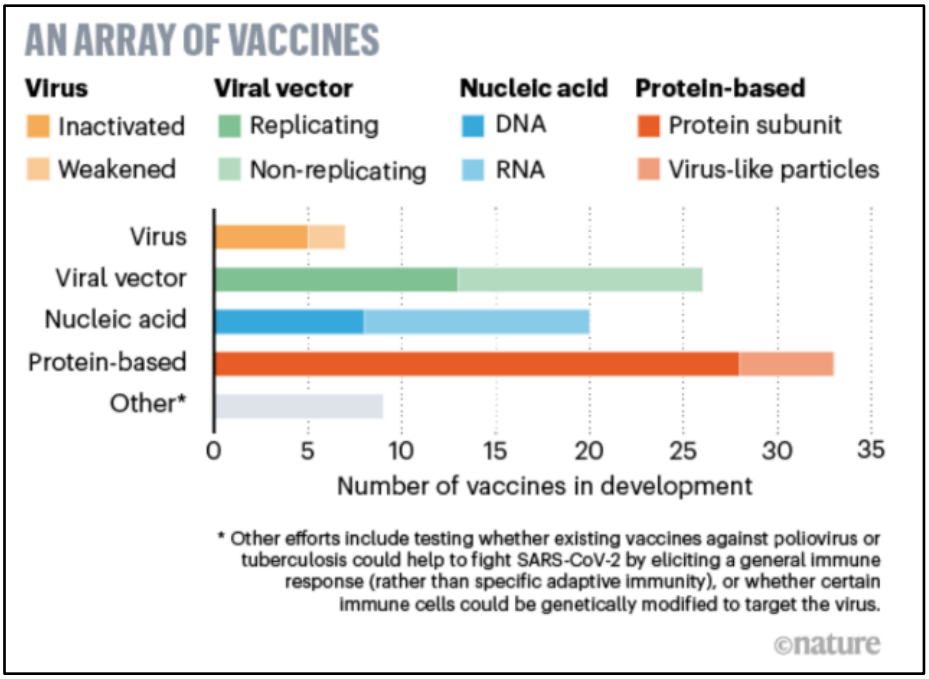

Fig. 2

Vaccine technical platforms.

Multiple technological platforms as well numerous minor variants of the same platform are exploited to induce an immune response to the target antigens. Just a few details of the most common technological platforms are reported here.

- Attenuated SARS-CoV-2. This is the most traditional technology exploited in the construction of vaccines. The attenuated virus, giving rise to a limited disease, generally elicits a strong immune response. Probably, only a minor part of the induced response is effective in preventing infection. Projects on attenuated SARS-CoV-2 are based on a technology well known at the Serum Institute of India. It would be interesting to know the route of administration of these vaccines. The oral route (as in the case of the Sabin polio vaccine) and the intranasal route could induce a mucosal immunity based on secretory IgA and IgM.

- Inactivated SARS-CoV-2. This too is a very traditional technological platform that has led to numerous vaccines in the past. The induced response is generally less strong than that induced by attenuated viruses, but the vaccine is more easily handled. The project carried out by Sinvac Biotech, China, has shown that the vaccine induces protective immunity in animals and is currently in Phase 1/2 in humans. A similar vaccine project carried out by the Wuhan Institute of Biological Products and Sinipharm, China is in early stage human test.
- Spike protein
  - Native Spike protein in its trimeric form is the antigen of the vaccine studied by the Clover Biopharmaceuticals Inc, Australia.
  - Spike protein admixed with AS03 adjuvant form the vaccine candidate of the joint project Sanofi, France, and GlaxoSmithKline (GSK), US, Italy and UK.
  - Subunits of the Spike protein obtained in different ways and combined with nanoparticles, adjuvants, CpG, spherical particles of the tobacco mosaic virus, the immunoglobulin Fc fragment, enclosed in outer membrane vesicles are the target antigens for over 40 vaccine projects. A potential vaccine studied at the Univ of Pittsburgh, US is based on subunits of Spike protein delivered into the skin by a fingertip-sized patch of 400 tiny needles.
  - *E. coli* engineered to express Spike and N proteins of the SARS-CoV-2 virus are administered orally in the vaccine candidate project in progress at MIGAL Galilee, Israel. This oral vaccine could elicit a mucosal immunity.
  - A pill containing different combinations of SARS-CoV-2 proteins is studied at Vaxart, US. This oral vaccine candidate could also elicit a mucosal immunity.
  - VLP expressing the Spike protein are studied by various groups (see above).

- DNA plasmid vaccines. DNA vaccines are based on the possibility of inducing the body cells to temporarily produce the protein against which an immune response should be induced. Once injected into the muscle, DNA plasmids coding for the Spike protein or its external fragments can spontaneously enter the cells, or their entry can be variously facilitated. DNA vaccination stimulates the production of antibodies but can lead to the activation of killer T cells. DNA and RNA vaccines have not yet been specifically tested on elderly people, the population with the greater need for an efficient vaccine for COVID-19. Over 9 vaccine projects are based on DNA plasmid coding fragments of the Spike protein.
    - One of these projects, leaded by Takis, Castel Romano, Italy exploits pieces of linear DNA instead of circular plasmids.
    - INO-4800 is an Inovio Pharmaceuticals, US, prepared plasmid coding for the Spike protein to be electroporated through the Celletra electroporator. Phase 1 human trial is underway.
    - DNA plasmids coding for the Spike protein electroporated through the clinical grade apparatus built by Igea, Carpi, Italy are studied at Karolinska Institutet, Sweden.
- mRNA based vaccines: RNA vaccines. RNA vaccines have been developed precisely to be produced in a very short time. The RNA specific for a particular protein is brought into cells by various methods. Once the RNA has penetrated the cells of the organism, the cells use its genetic information to produce the target protein.
    - RNA and lipososmes. RNA coding for SARS-CoV-2 proteins is brought into cells encapsulated by liposomes in the vaccine projects pursued by Moderna/NIAID, US; BioNTech/Fosun Pharma/Pfizer, Germany; BIOCAD, Russia; Univ Tokio/Daiichi-Sankyo, Japan; Fudan Univ/Shanghai Jiao Tong Univ/RNAcure, China; Cab Sino Biologics, China; Translate Bio/Sanofi Pasteur, US; Curevac, Germany. A novel lipid-mediated mRNA delivery system (LUNAR) is exploited by the US ArcturusTherapeutics.

- Moderna/NIAID/BARDA vaccine candidate project. On March 17, 2020, 76 days only since the sequence of SARS-CoV-2 was published, Dr. Michael Witte administered to volunteers the first shot of the mRNA-1273 candidate vaccine prepared by Moderna, a biotech company from Cambridge, MA. On May 18, 2020 Moderna reported that after two doses all participants across the 25 μg and 100 μg dose cohorts seroconverted with binding antibody levels at or above levels seen in convalescent sera. The vaccine elicited SARS-CoV-2 neutralizing antibody in all eight initial participants across the 25 μg and 100 μg dose cohorts, reaching or exceeding neutralizing antibody titers generally seen in convalescent sera[8].
- BioNTech/Fosun Pharma/Pfizer RNA vaccine is tested in human Phase1/2 trial.
- Intranasal mRNA. The Belgian eTheRNA is studying a mRNA vaccine administered intranasally in combination with other three naked mRNA molecules (TriMix technology) that stimulates dendritic cell activation. This vaccine could elicit a strong T cell immunity and a mucosal immunity.

- Virus based vaccines. A virus is genetically engineered so that it can induce the production of SARS-CoV-2 proteins in the body. There are two types of engineered viruses: those that are still able to replicate within human cells and those that cannot because a few key genes have been disabled. Non-replicating viral vectors carrying the gene for the Spike protein are exploited in over 13 vaccine projects, while replicating viral vectors are exploited in over 16 vaccine projects.
- A vaccine based on a non-replicating adenovirus vector carrying the Spike protein gene has been produced by CanSino Biologicals/Academy of Military Medical Sciences, China, and has already been tested in Phase 1 and Phase 2 clinical trials. On May 22, 2020, Zhu FC et al. reported on Lancet that one injection of this vaccine at all dose concentrations tested induced both specific antibody and T cell response. T cell response was evident 14 days after vaccination while antibody response peaked at day 28[9].

- The University of Oxford Jenner Institute, UK, non-replicating chimpanzee adenovirus vectored vaccine candidate ChAdOx1 nCov-19, carrying the Spike protein gene, started clinical trials. This candidate vaccine is produced in collaboration with ADVENT-IRBM (Pomezia, Italy) and AstraZeneca UK. 30 million doses of this candidate vaccine have already been purchased by the UK Government. The US Operation Warp Speed announced an investment of up to $ 1.2 billion in this vaccine project, noting that the delivery of the first of at least 300 million doses should arrive in October. The money comes from the US Biomedical Advanced Research and Development Authority (BARDA)[10]. An option relating to the acquisition of a substantial number of this vaccine candidate has also recently been put forward by the Italian government.
- Capitalizing on its successful experience from other vaccines, the biotech ReiThera - GSK, Castel Romano, Italy developed a vaccine candidate based on non-replicating chimpanzee adenovirus vector which will be tested in Phase 1-2 clinical trials in the coming weeks.

- *Bifidobacterium* probiotic engineered with the DNA encoding the Spike protein is studied at Symvivo, Canada. Phase 1 human trial is underway.
- Dendritic cells engineered to express SARS-CoV-2 proteins are studied at Shenzhen Geno-Immune Medical, China. Phase 1 human trial is underway.

June 8 2020, COVID-19 CANDIDATE VACCINES

PLATFORM	EXISTING VACCINES	DOSES	NUMBER OF PROJECTS	PHASE 1-2 CLINICAL TRIALS	
ATTENUATED VIRUS	Several	Single	2		
INACTIVATED VIRUS	Several	Multiple	5	4	Sinovac; Wuhan Inst; Sinopharm; Inst med Bio, China
PROTEIN	Several	Multiple	45	1	Novavx, USA
VIRUS LIKE PARTICLES	No	Multiple	9		
REPLICATING VIRAL VECTOR	SARS	Single	16		
NON REPLICATING VIRAL VECTOR	No	Multiple	15	2	CanSino, China Oxford/AstraZeneca, UK
DNA	No	Multiple	9	1	Inovio, USA
RNA	No	Multiple	15	2	Moderna; Pfizer, USA
OTHER	-	-	3		

Fig. 3. The vaccine list here reported is based on the WHO Draft Landscape of COVID-19 candidate vaccines https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines

3. Assessment of vaccine efficacy

The administration of the new vaccine on a limited number of volunteers, as is already the case with a few COVID-19 vaccines, makes it possible to understand whether the vaccine induces a good antibody response and / or a T cell response and whether its administration causes clear adverse events. Subsequently, the real evaluation of the effectiveness of the new vaccine will be based on randomized controlled trials that will compare the incidence of COVID-19 in groups of vaccinated and non-vaccinated people. Only the extension of this evaluation to progressively larger groups and for longer periods will determine whether one, several or none of the new COVID-19 vaccines protects effectively or only marginally and if its administration is associated with important collateral events.

Ethical issues. The race to develop one or more COVID-19 vaccine as quickly as possible not only has impressive economic aspects, not only is taking on political significance, but also opens up controversial ethical aspects. In fact, assessing the efficacy of a vaccine on groups of a population exposed to natural infection is a process that relentlessly takes a long time. As alternative, one can only rely on indirect surrogate markers of vaccine efficacy, such as induction of antibodies, specificity of the induced antibodies and T cell reactivity. The pressure induced by the pandemic may also lead to resuming controversial procedures based on the vaccination of volunteers who are subsequently deliberately infected with the SARS-CoV-2[11]. Support for this risky and highly controversial human vaccine-challenge study, with ethical implications is growing [12].

Risks associated to fast track vaccine evaluations. It is likely that, in view of the enormous pressure exerted by the COVID-19 pandemic, surrogate markers are initially used to decide whether the new vaccine could reasonably be employed for vaccination. These markers could include the evaluation of the amount of antibodies or the intensity of the reaction of the T killer cells induced by the vaccine on the volunteers. However, the administration of a new vaccine must always be carefully associated with a rigorous study of its safety. This is particularly important because a vaccine is not a drug for sick people at risk of dying, but rather a treatment that is given to those who are well so as to prevent the risk of falling ill[1].

The race to develop a COVID-19 vaccine is not only justified but absolutely necessary. However, the time required to evaluate the dangers and risks that may arise from a new vaccine must be included in its development. In some cases, vaccines prepared against other coronaviruses or other viruses have worsened the disease[13] and have induced T helper 2-type immunopathology[14]. These issues must be carefully evaluated and excluded before a new COVID-19 vaccine is distributed to combat the pandemic or its subsequent outbreaks.

4. Production and economic issues

Once the new vaccine has been validated, subsequent problems will be related to production and distribution. Technological, organizational, regulatory and economic problems will have to be overcome. The industrial technology needed to scale up the production to a billion doses will depend on which kind of vaccine works best. Initially it might not be physically possible to make enough vaccines for the world's population. In addition, political and economic constraints may limit vaccine access to the country that makes it or to the countries that can afford to pay for it. To make the new vaccines available to the global population will be challenging[15]. The WHO is trying to make sure that vaccine stockpiles are shared equitably, a crucial challenge that must be collectively addressed by governments[16].

Hence the consideration that vaccines for COVID-19, if effective, will be very difficult to be generally available before several months This long interval raises another problem of crucial importance: it is possible that by the time the vaccine arrives it will no longer be crucial or it will be exploited by only a small population in a particular area of the world. In fact, we cannot predict what the evolution of COVID-19 will be: the pandemic will end; the epidemic will continue to hit massively; it will only spread in some areas of the world; or there will be periodic outbreaks of new epidemics. In any case, the vaccine will be needed worldwide to boost COVID-19 immunity[4]. In this perspective of social responsibility and equitable sharing of preventive and therapeutic measures, readers are referred to an ad hoc position paper of the Accademia dei Lincei[17].

5. Other recommended vaccines and BCG

At present, no reliable data are available concerning the impact of seasonal influenza vaccination, anti-polio and anti-pneumococcus vaccines on the incidence and clinical progression of COVID-19. However, it should be underlined that we agree with the general recommendation of anti-pneumococcal vaccination in the elderly because of its effectiveness in

protecting against super-infection by pneumococcus in the course of viral infections and in reducing the appearance of bacteria resistant to antibiotics.
Lastly, somewhat connected with vaccines, it is worth mentioning the hypothesis that the old anti-tuberculosis Bacillus Calmette Guerin (BCG) vaccine may reduce the risk of SARS-CoV-2 infection. Two independent, not yet peer-reviewed epidemiological studies endorse this hypothesis showing an inverse relationship between COVID-19 attributable mortality and the country's policy concerning BCG vaccination[18,19]. A team in the Netherlands has launched a clinical trial with 1,000 health care workers. Similar trials in other countries will evaluate whether BCG vaccine increases resistance to SARS-CoV-2 in elderly people[20]. Innate immunity plays a key role in controlling the first stage of SARS-CoV-2 infection[21]. Therefore, strategies which increase innate immunity ("training strategies") need to be carefully evaluated by epidemiologists and in carefully controlled clinical studies [22].

6. Conclusions

The possibility of producing and distributing a salvific COVID-19 vaccine to a population of a nation is taking on a very important political value. Initiatives such as the US Operation Warp Speed and vaccine predatory attitudes shown by some nations are part of an international political game. A possible role that the Italian Government could play while leading the 2021 G20 meeting, to promote an equitable distribution of the first doses of a vaccine, is the topic of another document from Accademia Nazionale dei Lincei, which readers are specifically referred to.

July 2020

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