COVID-19 Vaccination

A thesis submitted in partial fulfillment of
the requirements of the Degree of
Master of Science in Advanced Clinical Pharmacy

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2021

Abstract

In 1796 Edward Jenner performed the world's first vaccination against smallpox. Since then, there have been many achievements in the field of vaccines including the recent development of the mRNA vaccines. The emergence of the novel SARS-CoV-2 virus and COVID-19 illness has created an urgent need for the development of safe and effective vaccines to protect against COVID-19.

The aims of this study were to 1) to review the available safety and efficacy literature of COVID-19 vaccines from selected databases and present the data in a comprehensible format and 2) to review the characteristics of the COVID-19 vaccines that are authorized for use in the European Union or under rolling review by the European Medicines Agency (EMA).

Fourteen studies were included for the review from literature search. The studies included mRNA vaccines, inactivated vaccines, chimpanzee adenovirus-vectored vaccine, recombinant adenovirus type-5-vectored COVID-19 vaccine, SARS-CoV-2 recombinant spike protein nanoparticle vaccine, protein subunit vaccine and recombinant adenovirus vaccine. The studies included adult participants both men and women ranging from the age of 16 and older. Various study group designs were used. Efficacy was measured in 11 studies via immunologic responses to vaccination including neutralizing antibodies against SARS-COV-2. Four studies measured the efficacy of the vaccines against COVID-19. Safety was commonly measured with local and systemic adverse reactions after vaccination. Adverse reactions commonly included pain and fever that were mild and transient. All the studies showed either immunologic response or efficacy against COVID-19 after vaccination.

The 4 COVID-19 vaccines that are authorized for use in the European union are the Comirnaty from Pfizer and BioNTech, mRNA-1273 from Moderna, AZD1222 from AstraZeneca and Ad26.COV.S from Janssen. Three vaccines are under rolling review of the

European Medicine Agency. These are the NVX-CoV2373 from Novavax CZ AS, CVnCoV from CureVac AG and the Sputnik V from Gamaleya.

More studies are needed to assess the safety and efficacy of the COVID-19 vaccines in special populations such as pregnant or breastfeeding women and children.

Acknowledgments

I would like to express my gratitude to my supervisor Prof. Anthony Serracino Inglott and co-supervisor Dr Nicolette Sammut Bartolo. Also, I would like to thank Prof. Lilian Azzopardi and all the staff from the department of pharmacy at University of Malta. A special thanks to my fellow students who made this journey so unforgettable.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

ACE 2	Angiotensin converting enzyme 2
DC	Dendritic cell
EMA	European Medicines Agency
FDA	Food and Drug Administration
HBV	Hepatitis B Virus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
MHRA	Medicines and Healthcare products Regulatory Agency
NSP	Non-structural proteins
OPV	Oral Polio Vaccine
ORF	Open-reading frame
RBD	Receptor-binding domain
SAM	Self-amplifying mRNA
US	United States
VLP	Virus-like Particles
VP	Viral particles
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1. History of vaccines

The invention of vaccines is one of the most important achievements in medical science. In the light of the COVID-19 pandemic and the race for the development of safe and effective vaccine against COVID-19, it is relevant to reflect about the history of vaccination. The beginning of modern vaccination (Figure 1) can be traced back to Edward Jenner's invention of the smallpox vaccine (Stern and Markel, 2005). The history of vaccination includes the tremendous work of many ingenious individuals and their contributions have brought us to the present state of medical science (Plotkin and Plotkin, 2011; Baicus, 2012; Hoenig et al, 2018). Some important achievements in vaccination are the invention of vaccine against rabies virus (Table 1.1) by Louis Pasteur, the first influenza vaccine, polio vaccines and the licensing of Albert Sabin's oral polio vaccine (Baicus, 2012; Hicks et al, 2012; Hannoun, 2013). With the advancements in vaccine technologies other important vaccines have become available such as the hepatitis B vaccine and human papilloma virus vaccine (Roldão et at, 2010; Harper and DeMars, 2017), which are examples of the many important breakthroughs that have happened in the field of vaccinations.[1]

[1] The College of Physicians of Philadelphia. The History of Vaccines: All Timelines Overview [Internet]. [cited 2021 Mar 2]. Available from URL: https://www.historyofvaccines.org/timeline/all

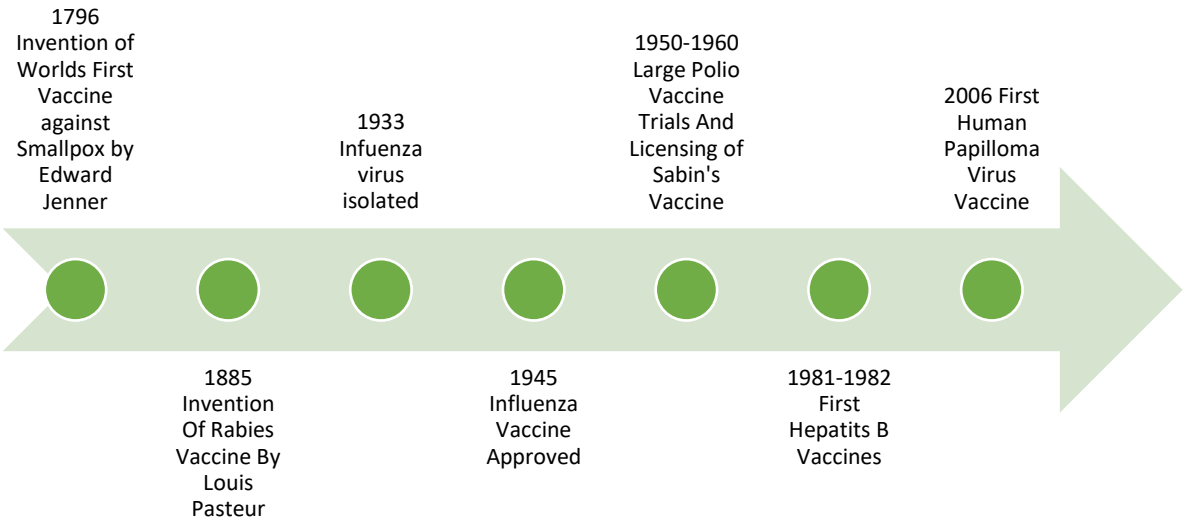

Figure 1: Selected timeline of vaccine development (adapted from *The College of Physicians of Philadelphia. The History of Vaccines: All Timelines Overview [Internet]. [cited 2021 Mar 2]. Available from URL: https://www.historyofvaccines.org/timeline/all)*

In 1796 the world's first vaccination was performed for protection against smallpox (Stern and Markel, 2005). This was done by a doctor named Edward Jenner who lived in England at the time and that area was subject to periodic smallpox outbreaks (Stern and Markel, 2005). Although today eradicated, smallpox was a serious health concern for people living prior to the era of vaccinations (Moore et al, 2006). The fatality of ordinary smallpox ranges from 10-60% and its more severe form, hemorrhagic smallpox, has a higher fatality rate of 93-100% (Moore et al, 2006). Smallpox is caused by a variola virus that belongs to the *Poxviridae* family and to the *orthopoxvirus* genus (Breman and Henderson, 2002). The *poxviridae* family consist of highly complex and large viruses and other viruses such as monkeypox, cowpox and vaccinia that belong to the same genus as the variola virus (Moore et al, 2006). In the days when Jenner invented the smallpox vaccine, it was known among people handling farm animals, that milkmaids who were exposed to symptomatic cowpox,

were immune to the effects of these smallpox outbreaks (Stern and Markel, 2005). This knowledge was the basis for Jenner to try replicating the phenomenon that would become the first vaccination and the first clinical trial for a vaccine in human history (Stern and Markel, 2005). The procedure included taking a sample of pus from a lesion caused by an unspecified poxvirus and injecting the sample subcutaneously to healthy subjects (Plotkin and Plotkin, 2011). Despite all the safety risks associated with such crude methods, the procedure was adopted all around the world and thus started the new era of vaccines (Plotkin and Plotkin, 2011). In 1967, The World Health Organization (WHO) intensified their monitoring and containment of smallpox around the world, which then led smallpox to be declared as eradicated in 1980 and no natural cases of smallpox have been reported since 1977 in Somalia.[2]

Rabies is a serious disease that is most commonly caused by the Rabies Virus that is a prototype virus of the *Lyssavirus* genus and it is usually transmitted to humans via bite of another mammal, most often by a dog. (Fooks et al, 2017). In France after multiple years of experiments, a microbiologist called Louis Pasteur, with his team, successfully developed the first effective vaccine against rabies (Stern and Markel, 2005; Hicks et al, 2012). The first human subject to receive the vaccine was a boy who had been bitten by an infected dog 60 hours prior to the administration (Wu et al, 2011). This vaccine was prepared from rabies infected rabbit's spinal cord that was air-dried and emulsified. Multiple injections were given to the boy and estimated total of 47 ml was administered (Wu et al, 2011). This method of rabies vaccination was continued for the next 50 years with no major changes to the preparation (Wu et al, 2011).

[2] World Health Organization (WHO). Smallpox [Internet]. [cited 2020 Dec 3]. Available from URL: https://www.who.int/health-topics/smallpox

The discovery of Pasteur led to people from around the world to travel to France to receive treatment after being bitten by a rabies infected animal (Hoenig et al, 2018). In 1888 the Pasteur institute was founded to spread the rabies vaccination program and to function as a non-profit scientific organization.[3] An American doctor named Valentine Mott was a major influence in establishing the first Pasteur institute into United States (US) and performed the first rabies vaccination in the country (Hoenig et al, 2018). The first human subject in US to receive the rabies vaccine that was made from rabbit's spinal cord was a boy named Harold Newton who had been bitten by their pet dog that was infected with rabies. (Hoenig et al, 2018).

Another notable development is the vaccine against poliomyelitis. Poliomyelitis is an acute paralytic disease caused by a poliovirus that is part of the *Picornaviridae* family (Baicus, 2012). Polio is extremely infectious and is transmitted commonly by the fecal-oral route from person to person.[4] The history of polio can be traced back as early as the times of ancient Egyptians and the first epidemic is reported to be in Stockholm in 1887 (Baicus, 2012). Another significant polio outbreak happened in 1916 in United States where the virus was found to spread by asymptomatic carriers resulting in 6,000 people dead and 27,000 paralyzed (Baicus, 2012). The severity of this, and future epidemics, led to more investigation into the disease and successful culturing of the virus. After unsuccessful attempts to develop safe polio vaccines by Brodie and Kollmer (Baicus, 2012), Jonas Salk was able to produce an inactivated polio vaccine which he tested on his own family in 1953.[1] Salk developed the vaccine consisting of three virus strains by using infected kidneys of a monkey and then inactivated the virus by formalin treatment (Baicus, 2012). The United States, Finland and

[3] Institut Pasteur. History [Internet]. Paris: Institut Pasteur; 2017 [cited 2020 Dec 3]. Available from URL: https://www.pasteur.fr/en/institut-pasteur/history

[4] World Health Organization (WHO). Poliomyelitis [Internet]. WHO; [cited 2021 Mar 26]. Available from URL: https://www.who.int/health-topics/poliomyelitis#tab=tab_1

Canada participated in the clinical trial of this vaccine in 1954 (Monto, 1999; Marks, 2011). This large trial included a total of 432,217 children who were vaccinated. The trial was a success and showed efficacy of 70% with 95% confidence interval with the endpoint being paralytic poliomyelitis (Monto, 1999). Although successful, the Salk's vaccine was not without its limitations as the production of the vaccines required 1500 monkeys per million doses, resulting in obvious logistical and ethical implications (Baicus, 2012). Later Albert Sabin developed a trivalent oral vaccine that led to good antibody levels and was not as neurotropic as the previous ones (Baicus, 2012). Sabin's vaccine was licensed in 1961-1963 in the US. The vaccine included three live attenuated strains: P1/Lsc,2ab, P2/ P712,Ch,2ab and P3/Leon,12a1b (Sabin et al, 1960; Baicus, 2012). Sabin eventually donated his strains to WHO, which increased the number of children vaccinated from 5% to 80% by the year 1995 (Baicus, 2012). The oral polio vaccine (OPV) was not without its risks though as the OPV could go through genetic mutation in the human intestine, leading to Vaccine-Associated Paralytic Poliomyelitis cases (Baicus, 2012). Since 1988, the natural polio cases have decreased by 99% to only 175 cases by 2019.[4]

Table 1.1: Selected vaccine preventable diseases

Disease	Causative pathogen / strain(s)	Main symptoms	References
Smallpox	Variola virus	Fever, rash, focal lesions, sepsis, pneumonia, encephalitis, corneal ulcers and death.	Moore et al, 2006
Rabies	Rabies virus	Hydrophobia, excess salivation, intermittent agitation, muscle weakness, paralysis, coma and death.	Fooks et al, 2017
Poliomyelitis	Polio virus	Paralytic symptoms, headache and death.	[5]
Influenza	H1N1, H2N2, H3N2 and B-strain	Fever, cough, sore throat, muscle aches and headaches.	[6]
Hepatitis B	Hepatitis B virus	Jaundice, dark urine, fatigue, nausea, vomiting and stomach pain.	[7]
Cervical Cancer	Human papilloma virus	Unusual bleeding, pain, discomfort, unusual vaginal discharge and lower back pain.	[8]

[5] Centers for Disease Control and Prevention (CDC). What is Polio? [Internet]. CDC; Last updated: October 24, 2019 [cited 2021 Mar 26]. Available from URL: https://www.cdc.gov/polio/what-is-polio/index.htm
[6] Centers for Disease Control and Prevention (CDC). Flu Symptoms & Complications [Internet]. CDC; Last updated: February 24, 2021 [cited 2021 Mar 1]. Available from URL: https://www.cdc.gov/flu/symptoms/symptoms.htm
[7] World Health Organization (WHO). Hepatitis B [Internet]. WHO; 2020 [cited 2021 Mar 1]. Available from URL: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b
[8] National Health Service (NHS). [Internet]. Cervical cancer. NHS; Last updated: May 11, 2018 [cited 2021 Mar 1]. Available from URL: https://www.nhs.uk/conditions/cervical-cancer/symptoms/

Influenza remains a serious health condition worldwide (Roldão et at, 2010). Severe forms of the illness adding up to 3-5 million cases worldwide of which 290,000-650,000 cases results in death.[9] Influenza viruses are part of the *Orthomyxoviridae* family (Taubenberger and Kash, 2010). The first influenza vaccine was developed after the successful isolation of the influenza virus (H1N1) from ferrets in 1933 (Hannoun, 2013). The vaccine contained a single influenza A virus strain. Shortly after a second different influenza strain (B) was discovered. This consequently led to the development of a bivalent influenza vaccine (Hannoun, 2013). The modern vaccines are either inactivated or attenuated influenza vaccines.[10] Both of these vaccine types commonly contain three different viral strains: H3N2, H1N1 and one B strain. More recently quadrivalent vaccines have become also available.[10]

Chronic hepatitis B affects 370 million people worldwide (Roldão et at, 2010). Hepatitis B is a serious infection that may lead to liver cirrhosis, cancer and eventually to death (Roldão et at, 2010). The hepatitis B virus (HBV) is structured as a double stranded DNA surrounded by envelope proteins (Tiollais et al, 1985). The first vaccines for HBV were developed by Merck & Co., Inc and Institut Pasteur, Paris, France between 1981 – 1982 (Roldão et at, 2010). These vaccines were developed from inactivated HBsAg particles obtained from recovered hepatitis B patient's plasma. As there are risks in using blood products especially in large scale, an alternative source was needed for future vaccine development (Roldão et at, 2010). To produce recombinant hepatitis B virus like particles, yeast cells: *Saccharomyces cerevisiae*, *Pichia pastoris* and *Hansenula polymorpha* as well as chinese hamster ovary cells (CHO) are used (Roldão et at, 2010).

[9] World Health Organization (WHO). Influenza (Seasonal) [Internet]. WHO; [cited 2020 Dec 8]. Available from URL: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)
[10]World Health Organization (WHO). Types of seasonal influenza vaccine [Internet]. WHO; [cited 2020 Dec 7]. Available from URL: https://www.euro.who.int/en/health-topics/communicable-diseases/influenza/vaccination/types-of-seasonal-influenza-vaccine

Human papilloma viruses (HPV) are widely found on the skin of humans and other animals (Roldão et at, 2010). Although, over 100 different types of HPV are capable of infecting humans, most of them are asymptomatic. Less serious symptoms include lesions and warts. However, some subtypes of HPV, are capable of causing cervical cancer (Crosbie et al, 2013). The HPV vaccines consist of synthetic virus-like particles (VLP) (Harper and DeMars, 2017). VLPs are multiprotein structures that resemble the natural virus that they aim to immunize against and they lack the viral genome therefore having potential to be safer and more affordable in vaccine development (Roldão et at, 2010) The first HPV vaccine to be granted a marketing authorization was Gardasil in 2006 (Harper and DeMars, 2017). This is a quadrivalent vaccine protecting against HPV types: 6, 11, 16 and 18. The proteins were manufactured using specifically designed *Saccharomyces cerevisiae* yeast strain for each protein (Roldão et at, 2010). The L1 proteins develop in their own batches until the cells are destroyed and the proteins purified from other biological material. These proteins are then combined into a sterile suspension. Other available HPV vaccines are Cervarix and the more immunogenic version of Silgrad (also marketed as Gardasil), the Gardasil 9.[11]

1.2. The science and development of vaccines

There have been significant developments in the technologies utilized for vaccine development since Jenner performed his initial vaccination.[12] This has resulted in a variety of different types of available vaccines which have their own advantages and disadvantages. It has not been the case that the new technologies have replaced the older ones but rather

[11] National Cancer Institute. Human Papillomavirus (HPV) Vaccines [Internet]. National Cancer Institute. Last updated: September 9, 2019 [cited 2021 Mar 1]. Available from URL: https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-vaccine-fact-sheet

[12] U.S. Department of Health & Human Services (HHS). Vaccine Types [Internet]. HHS; Last updated: March 2021 [cited 2021 Mar 26]. Available from URL: https://www.vaccines.gov/basics/types

have expanded the toolkit for fighting against diseases. This is seen especially in the development of COVID-19 vaccines as many different vaccine technologies have been utilized in their development.[13] This section discusses the different types of vaccines that have been developed against various diseases.

1.2.1. Live-attenuated vaccines

Injecting live viruses without any preparation into human subjects, would lead to people getting very sick depending on the virulence of the virus. Therefore, since the early days of vaccination, live viruses have been weakened (attenuated) with various methods before administration (Plotkin, 2003). These first methods that were used for virus attenuation included heat and different chemical treatments for the viral material. The early attenuation attempts by Pasteur of drying the virus was not sufficient to produce consistently weakened strains (Hicks et al, 2012). In the case of the rabies vaccine, this led to using infected brains of sheep to extract the viral material and then using acidic phenol for chemical attenuation (Hicks et al, 2012). While solving the problem of consistent attenuation, this method of vaccine development resulted in residues, such as myelin, in the preparation and the impurities have the potential to cause adverse reactions leading to fatal encephalitis in the worst cases (Hicks et al, 2012). Another method used for both rabies and yellow fever vaccine was to use chick embryos for culturing weakened virus strains (Plotkin, 2003; Minor, 2015). Using inactivated infected mouse brains was also an option for vaccine development although, not recommended by the WHO as nervous tissue still can cause major adverse reactions (Hicks et al, 2012). Cell cultures derived from human diploid cell line have been

[13]Regulatory Affairs Professionals Society (RAPS). COVID-19 vaccine tracker [Internet]. RAPS; 2021 [cited 2021 Feb 22]. Available from URL: https://www.raps.org/news-and-articles/news-articles/2020/3/covid-19-vaccine-tracker

used as well (Minor, 2015). More recent methods for virus attenuation include virus cleavage site modifications, mRNA mediated attenuation and other genomic modifications (Jang and Seong, 2012). Whichever method used for the attenuation, the virus has to be able to continue replication within the vaccinated person (Hamborsky et al, 2015). A small amount of the attenuated virus is usually required to produce immunogenic response in the host which can cause similar symptoms as the disease itself, while being significantly milder (Hamborsky et al, 2015).

1.2.2. Inactivated vaccines

In contrast to attenuated vaccines, inactivated vaccines consist of killed virions and therefore, they cannot replicate in the vaccinated person (Hamborsky et al, 2015). Inactivated vaccines have to be administered in multiple doses to be able to produce a sufficient immunological response (Sanders et al, 2014). The first dose usually just primes the immune system and the second or third dose is what finally creates the protection (Hamborsky et al, 2015). Inactivated vaccines are produced by culturing the viruses or bacteria and then inactivating them with heat or chemicals (Sanders et al, 2014). Formalin is one of the earliest chemicals used for virus inactivation and has been used for the development of typhoid, cholera, influenza and hepatitis A vaccines (Plotkin, 2003). As of now, polio, hepatitis A, and rabies are available as whole-cell inactivated viruses (Hamborsky et al, 2015).

1.2.3. Subunit vaccines

In comparison to both inactivated and attenuated vaccines, subunit vaccines contain only those parts of an organism that are needed to produce an immunological response (Moyle

and Toth, 2013). This approach requires more comprehensive prior understanding of the target organisms and its components. It must also be determined whether the immunological response against the subunits of the pathogen is adequate to provide protection against the whole organism. Subunit vaccines usually contain an adjuvant in addition to the immunogenic subunit (Bali and Rafi, 2011). Adjuvants are compounds that are added to the vaccine to modify or increase the immune response of the vaccine. A commonly used adjuvant is aluminum salt (Alum).[14] These aluminum salts include potassium aluminum sulphate, aluminum hydroxide and aluminum phosphate. Another category of adjuvants include different oil-water based compounds such as MF59 and AS03 (Moyle and Toth, 2013). There are advantages of developing subunit vaccines compared to using whole organisms (Hansson et al, 2000). These advantages include the known composition, high production consistency of the immunogenic subunits and the possibility of producing vaccine that do not require cold storage (Hansson et al, 2000; Moyle and Toth, 2013). This group of vaccines has also its downsides as they often produce a weaker immune response and therefore require the use of adjuvants (Hansson et al, 2000).

1.2.4. Recombinant and polysaccharide vaccines

Recombinant vaccines are developed via modern DNA techniques where the genetic code of a selected subunit of a pathogen is inserted into culturing cells where the DNA is then translated.[15] Many cell lines can be used for producing recombinant vaccines such as yeast, bacterial, insect and mammalian (Nascimento and Leite, 2012). Which culture one chooses

[14] British Society for Immunology. Alum (1926) [Internet]. [cited 2020 Dec 28]. Available from URL: https://www.immunology.org/alum-1926
[15]Nature. Recombinant vaccine [Internet]. [cited 2020 Dec 28]. Available from URL: https://www.nature.com/subjects/recombinant-vaccine

to produce recombinant vaccines depends on the characteristics of the subunits. For example, if not much post-translational processing is required for the protein, a bacterial cell line can be used (Nascimento and Leite, 2012). If modifications such as glycosylation is required for the end product, a mammalian or insect cell line is preferred (Cox, 2012; Nascimento and Leite, 2012). As yeast cells are eukaryotic like human cells, they can be used in some cases where post-translational processing is required (Nascimento and Leite, 2012).

Some bacteria have highly virulent polysaccharides on their surface that protect them against phagocytosis (Parween et al, 2019). Against some of such bacteria, polysaccharide vaccines have been developed (Nair, 2012). Polysaccharide vaccines are subunit vaccines consisting of long carbohydrate chains (Hamborsky et al, 2015). These carbohydrate chains are similar to the surface polysaccharides of pathogenic bacteria and therefore produce an immunological reaction against the pathogen (Hamborsky et al, 2015). The immune reaction against these vaccines is not mediated via a T-cell response but instead the antigen induces a B-cell response (Nair, 2012; Hamborsky et al, 2015). The immune response for polysaccharide vaccines cannot be boosted by giving multiple doses (Hamborsky et al, 2015).

1.2.5. Conjugate and toxoid vaccines

Polysaccharide vaccines work by inducing a B-cell response toward the antigen and do not usually elicit a T-cell response (Nair, 2012; Hamborsky et al, 2015). Conjugate vaccines have been developed to produce a T-cell response against bacteria with the target surface polysaccharides (Hamborsky et al, 2015). This is important as in the case of young children, the B-cell response is not sufficient to produce adequate immune response against certain bacteria (Goldblatt, 2000). Conjugated vaccines are produced by attaching a protein carrier to the polysaccharide which makes it possible for the T-cells to recognize the antigen and to

produce an immunologic memory in younger children against pathogens such as *Haemophilus influenzae* (Goldblatt, 2000; Hamborsky et al, 2015). Multiple carrier proteins are available for development of conjugated vaccines such as a genetically modified cross-reacting material of diphtheria toxin, tetanus toxoid, meningococcal outer membrane protein complex, diphtheria toxoid, and *H. influenzae* protein D (Pichichero, 2013).

Toxoid vaccines are unique in that they target specifically the toxin that is produced or part of a bacteria instead of the organism as a whole.[16] Toxoid vaccines have been developed against diphtheria and tetanus. Toxoid vaccines are produced by inactivating the toxin with formalin (Jones et al, 2008). The inactivation process with formalin (0.2–0.6%) often takes around 7 days in a temperature of 30 °C, leading to the toxoid being weakened to the point of not causing a disease but is still recognizable to the immune system and result in immunity (Jones et al, 2008).

1.2.6. mRNA vaccines

One of the most recent advances in vaccinations is the use of messenger RNA (mRNA) vaccine technology (Pardi et al, 2018). mRNA is a piece of genetic code that is normally transcribed according to DNA and will act as a template for protein synthesis (Cooper, 2019). The translation happens inside the cell and within the ribosome which in eukaryotic cells consist of two subunits: the 60S and 40S. Once the mRNA has attached to the ribosome, transfer RNA (tRNA) attaches to the mRNA template (Griffiths et al, 2000). This leads to the chemical bonding of the subsequent amino acids carried by the tRNA. Once this process

[16] World Health Organization (WHO). Vaccine Safety Basics: Toxoid Vaccines [Internet]. [cited 2021 Mar 26]. Available from URL: https://vaccine-safety-training.org/toxoid-vaccines.html

is done, the protein's amino acid sequence is ready and it can be released to perform its function or further processed (Cooper, 2019).

Unlike in physiologic protein synthesis where the mRNA is transcribed within the cell, the mRNA vaccines require the mRNA to be delivered in the cytosol through the cell's phospholipid membrane (Pardi et al, 2018). There are currently two main delivery methods for mRNA vaccines. In the *ex vivo* loading of dendritic cells (DC), the DCs are extracted and loaded with the mRNA and then re-infused to the body (Pardi et al, 2018). The intake of mRNA by the DCs can be further increased with electroporation (Zhang et al, 2019). The other delivery method is a direct parenteral injection of the mRNA into the body. This process can also be enhanced by attaching additional components such as gold particles, polymers and lipid nano particles to the mRNA (Pardi et al, 2018).

Two main types of mRNA vaccines are the self-amplifying mRNA (SAM) vaccines and non-replicating mRNA vaccines (Pardi et al, 2018; Zhang et al, 2019). The SAM's genome coding the RNA replication machinery is based on alphavirus and the parts that code the specific proteins can be engineered according to need (Pardi et al, 2018; Bloom et al, 2020). The benefit of SAMs is the possibility of producing a massive number of antigens with very low dose of the vaccine itself and that SAMs do not necessarily need separate adjuvants as their own double-stranded RNA will produce similar results (Pardi et al, 2018; Scorza and Pardi, 2018). The limitations of SAMs include the difficulty of adjusting the inflammation and reactogenicity associated with these vaccines (Pardi et al, 2018). Non-replicating mRNA vaccines in contrast to the SAMs only contain the genetic information for coding the target antigen (Pardi et al, 2018). As the non-replicating RNA vaccines do not code their own replication machinery, they usually require larger doses as well as highly efficient delivery systems to produce adequate number of antigens (Scorza and Pardi, 2018).

mRNA vaccines are hoped to accomplish what the traditional vaccines have not been able to. Mainly, to tackle difficult infectious micro-organisms such as human immunodeficiency virus (HIV), Ebola, Zika and more recently the SARS-CoV-2 (Pardi et al, 2018; Poland et al, 2020). In addition, the mRNA vaccines could provide protection against various cancers (Pardi et al, 2020). They also offer the possibility of very rapid development, high potency and lower manufacturing cost. As with all vaccines, mRNA vaccines do cause side-effects such as injection site reactions and other adverse reactions typical to vaccines (Pardi et al, 2018).

1.3. Biology of SARS-CoV-2

Coronaviruses are part of the *Nidovirales* order which are enveloped, non-segmented positive-sense RNA viruses (Fehr and Perlman, 2015). Members of this order have very large genomes compared to other viruses. The genomic organization of coronaviruses don't experience lots of mutations and are therefore very conserved (Fehr and Perlman, 2015). The SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA β-coronavirus with its envelope being coated with spike glycoprotein, envelope and membrane proteins (Cevik et al, 2020; Gordon et al, 2020). Its diameter is between 60 – 140 nm and the spike proteins are around 9 – 12 nm (Wiersinga et al, 2020) The SARS-CoV-2 has a 30-kb genome that includes 14 open-reading frames (ORFs) (Gordon et al, 2020). These ORFs (ORFa and ORFab) are coding polyproteins that are broken into 16 non-structural proteins (NSPs). The replicase–transcriptase complex is built from these NSPs (Gordon et al, 2020). The replicase-transcriptase complex is the machinery responsible for coordinating the RNA synthesis for genome replication and transcription (Ulferts et al, 2009). The genes for coding the spike, envelope and -membrane proteins are found in the 3'end of the genome (Gordon et al, 2020).

The crucial structure of SARS-CoV-2 for infecting cells is its spike protein (Huang et al, 2020). The spike protein has a S1 subunit that is able to bind to the peptidase domain of angiotensin-converting enzyme 2 (ACE 2) (Huang et al, 2020). After getting inside the cell, the virus releases its viral genome to be translated by the host's ribosome (Cevik et al, 2020). The translated proteins form the replicase transcriptase complex which then participates in the following RNA replication and protein synthesis (Cevik et al, 2020). The further processing of the translated protein is done by three major enzymes 3CLpro, PLpro, and RdRp (Naqvi et al, 2020). Once the viral genome is replicated and the assembly of the new virions is done, it is released to extracellular space where it can infect other cells (Cevik et al, 2020).

1.4. Origins of SARS-CoV-2

The first cases of the novel coronavirus infections (SARS-CoV-2) were reported on December 29 2019 (Li et al, 2020). The origin for all four cases was most likely at Huanan seafood wholesale market in Wuhan China. After the emergence of the SARS-CoV-2, there are now a total of seven coronaviruses that are known to infect humans (Rabaan et al, 2020). Only SARS-CoV-1, SARS-CoV-2 and MERS cause serious symptoms in contrast to the other four: HKU1, NL63, OC43 and 229E (Andersen et al, 2020). The SARS-CoV-2 has strongest genetic similarity to SARS-CoV-1 and a bat coronavirus RaTG13 (Cevik et al, 2020). Multiple theories have been proposed about the origins of the SARS-CoV-2 including natural and laboratory origins (Andersen et al, 2020).

One possible origin is evolutionary selection before the virus first transmitted to humans (Lundstrom et al, 2020). It could be that the virus had evolved in bats as it has a 97.41% genetic similarity with the RaTG13 in key residues of the receptor-binding domain (RBD)

and O-linked glycan (Malaiyan et al, 2020). However, the spike protein of the SARS-CoV-2 is more adapted to binding human ACE2 receptors compared to RaTG13 (Andersen et al, 2020). There is a strong similarity of the spike protein between SARS-CoV-2 and a coronavirus found in Malayan pangolins (*Manis javanica*) that are sold in wet markets in China, but the overall genetic profile is still closer to the bat derived RaTG13 (Andersen et al, 2020; Lam et al, 2020). One major feature differentiating the SARS-CoV-2 from both bat and pangolin derived coronaviruses is the polybasic furin cleavage site found in SARS-CoV-2 (Andersen et al, 2020). It is found from the two subunits S1 and S2 of the spike protein. This polybasic cleavage site might be a significant contributor to the infectivity of the SARS-CoV-2 (Örd et al, 2020).

It could also be, that the evolutionary changes in the SARS-CoV-2 progenitor happened after a zoonotic transmission to humans, which would explain the absence of certain features in its animal counterparts (Andersen et al, 2020; Lundstrom et al, 2020). This would suggest that there had been sufficient transmissions of the progenitor virus between humans prior to the emergence of the SARS-CoV-2. As coronaviruses are studied in laboratories and leakages from these laboratories sometimes occur, it is possible the origin of the changes can be traced in such settings (Andersen et al, 2020). It is possible that the mutations might have happened as the virus adapts to cell cultures that are used in laboratories although such scenario is unlikely (Andersen et al, 2020; Lundstrom et al, 2020).

1.5. Virulence and pathogenesis of SARS-CoV-2

SARS-CoV-2 is most commonly transmitted via droplets of an infected person (Wiersinga et al, 2020). The greatest risk for transmission is when being face to face within 2 meters from infected person for about 15 minutes (Cevik et al, 2020; Wiersinga et al, 2020).

Coughing, talking and sneezing are significant factors in transmission. SARS-CoV-2 can also be transmitted by touching surfaces that contain the virus (Wiersinga et al, 2020). Hard surfaces such as steel and plastic can be sources for infection for up to 72 hours whereas permeable surfaces such as cardboard are not as likely to spread the infection (Wiersinga et al, 2020). The SARS-CoV-2 is more persistent in conditions with low temperature and low relative humidity (Aboubakr et al, 2020).

The SARS-CoV-2 infection produces a highest viral load at the time the first symptoms appear with viral shedding starting 2-3 days prior the emergence of symptoms (Cevik et al, 2020; Wiersinga et al, 2020). This is further enhancing the transmissibility compared to SARS-CoV-1, which has maximum viral load weeks after symptom onset (Cevik et al, 2020). The virus can be detected from the upper respiratory tract up to 83 days after symptom onset with mean of 17 days. The extend by which asymptomatic patients can spread the virus is still unclear (Wiersinga et al, 2020)

Commonly the SARS-CoV-2 infects nasal and bronchial epithelial cells and pneumocytes (Wiersinga et al, 2020). Early infection can lead to significant lymphopenia which relates to the disease severity as the virus kills T lymphocyte cells (Tan et al, 2020; Wiersinga et al, 2020). As the virus continues to replicate, the epithelial-endothelial barrier in the respiratory track can break up (Wiersinga et al, 2020). Eventually, a strong immune rection via monocytes and neutrophils leads to edema followed by the formation of scar tissue in the respiratory tract (Mason, 2020; Wiersinga et al, 2020). Due to massive inflammation in the lungs of severely ill patients, COVID-19 can result in microthrombi formation, which can affect coagulation elsewhere in the body (Wiersinga et al, 2020). This can lead to cardiac injury, deep venous thrombosis, limb ischemia, in addition to more local pulmonary embolism (Wiersinga et al, 2020; Bois et al, 2021).

1.6. COVID-19 symptoms

The average period from catching SARS-COV-2 to developing symptoms ranges from 5 to 6 days although, the symptoms might appear as late as after 14 days from infection.[17] Hospitalized COVID-19 patients most commonly present with fever, dry cough, shortness of breath, fatigue, muscle pains, nausea and gastrointestinal problems such as vomiting or diarrhea (Wiersinga et al, 2020). However, the clinical presentation of COVID-19 can vary and other symptoms such as temporary loss of smell and taste due to damage it causes in the olfactory epithelium (Cevik et al, 2020). COVID-19 may cause complications in multiple other organ systems in addition to the respiratory system (Wiersinga et al, 2020). The ACE2, which is the receptor SARS-CoV-2 uses to infect cells, is widely expressed in cardiovascular system (Talasaz et al, 2020). Infection of heart tissues may lead directly to myocarditis in addition to the cardiovascular risks caused by the high inflammation associated with COVID-19 (Talasaz et al, 2020). It is also, suggested that SARS-CoV-2 might cause neurological and psychiatric problems.[18] It appears that the commonly reported neurological symptoms e.g., headache, dizziness and impaired consciousness, might not be caused by SARS-CoV-2 direct infiltration to the nervous system but could be due to the immune reaction (Chen et al, 2020). Renal complications have also been reported in hospitalized COVID-19 patients with most common reported renal complication being hyperkalemia and acute kidney injury being the second (Kunutsor and Laukkanen, 2020).

[17] World Health Organization (WHO). Transmission of SARS-CoV-2: implications for infection prevention precautions [Internet]. WHO; 2020 [cited 2020 Dec 30]. Available from URL: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions

[18] Marshall M. How COVID-19 can damage the brain [Internet]. Nature Publishing Group; 2020 [cited 2020 Dec 31]. Available from URL: https://www.nature.com/articles/d41586-020-02599-5

1.7. Mortality of COVID-19

The overall mortality rate of COVID-19 varies somewhat between countries. According to the European Centre for Disease Prevention and Control, between 31 December 2019 and 14 December 2020 the worldwide mortality rate is ~2.26% with a total of 71,503,614 cases from which 1,612,833 cases resulted in death.[19] In the US since 21 January 2020 until 16 December 2020 the total of COVID-19 cases is 16,317,892 from which 300,032 have resulted in death.[20] According to this data the mortality rate of COVID-19 is around 1.84% in the US. However, the overall mortality rate might not yet be clear as the reporting criteria for COVID-19 deaths and the method of calculating differs between entities.[21] Case fatality ratio is calculated by dividing the number of deaths from COVID-19 with the number of confirmed cases of the disease which slightly differs from using infection fatality ratio, where the denominator is the number of infected patients.[21]

1.8. Long-term effects and risk factors of COVID-19

In addition to the effects of the irreversible scarring of pulmonary tissues, the COVID-19 disease has been proposed to cause long-term symptoms lasting for months after the initial infection (Ludvigsson, 2020). It has been reported that non-specific symptoms such as fatigue, shortness of breath, heart palpitations and chest pain may persist up to 2 months after being diagnosed with COVID-19 (Ludvigsson, 2020). Also, persistent neurological problems

[19] European Centre for Disease Prevention and Control (ECDC). COVID-19 situation update worldwide, as of 14 December 2020 [Internet]. Last updated. December 14, 2020 [cited 2020 Dec 16]. Available from URL: https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases

[20] Centers for Disease Control and Prevention (CDC). CDC COVID Data Tracker [Internet]. [cited 2020 Dec 16]. Available from URL: https://covid.cdc.gov/covid-data-tracker/

[21] World Health Organization (WHO). Estimating mortality from COVID-19 [Internet]. WHO; 2020 [cited 2020 Dec 31]. Available from URL: https://www.who.int/news-room/commentaries/detail/estimating-mortality-from-covid-19

are possible due to the hypoxia caused by severe forms of the disease (Kantonen et al, 2020). It is likely that COVID-19 will have prolonged effects of the immune system as well.[22] In the case of SARS-CoV-1, the production of interferons is suppressed after infection, therefore weakening the host's immune system (Hu et al, 2020). Given the immune suppression, it is possible that COVID-19 could make patients more susceptible to further infections.

Most cases of COVID-19 are considered mild or moderate (Jordan et al, 2020). However, 14% of the cases present with severe symptoms and 5% of the patients develop into critical state (Jordan et al, 2020). Multiple risk factors for developing the more severe illness have been identified, with some being non-modifiable and others being modifiable with e.g., lifestyle changes (Ho et al, 2020). Modifiable risk factors for severe COVID-19 include smoking, obesity, bad lung function, treated and non-treated hypertension, slow walking pace and low HDL levels (Ho et al, 2020). Non-modifiable risk factors include baseline diabetes, chronic pulmonary disease, cardiovascular disease, general long-standing illness, older age, black ethnicity, male sex, socioeconomic deprivation, sleep apnea, elevated white blood cell count and higher cystatin C (Ho et al, 2020; Shi et al, 2020).

[22] Marshall M. The lasting misery of coronavirus long-haulers [Internet]. Nature Publishing Group; 2020 [cited 2020 Dec 31]. Available from URL: https://www.nature.com/articles/d41586-020-02598-6

1.9. Aims of the study

The aims of the study were:

1. To review the available safety and efficacy literature of COVID-19 vaccines from selected databases and present the data in a comprehensible format.
2. To review the characteristics of the COVID-19 vaccines that are authorized for use in the European Union or under rolling review by the European Medicines Agency (EMA).

CHAPTER 2

METHOD

2.1. Methodology overview

The study was divided into two phases. The first phase entailed a literature review related to the safety and efficacy of COVID-19 vaccines. In the second phase of the study a review was done for the COVID-19 vaccines that are authorized for use in the European Union or are under rolling review by the European Medicines Agency.

2.2. Review of published clinical trials on COVID-19 vaccines

This part of the study aimed to review the available literature about the safety and efficacy of COVID-19 vaccines. The review included participants demographics, study methods including group designs and outcome assessments. Main results from clinical trials were also reviewed. A literature search was conducted for this aim. Three different databases were chosen for the search: PubMed, Cochrane Central Register of Controlled Trials (EBSCO) and Medline ProQuest. The search string: *(covid-19 OR sars-cov-2) AND (vaccine OR vaccines) AND (efficacy OR safety)* was used for all three databases.

The inclusion criteria for the studies were:

- Studies assessing the safety and/or efficacy of COVID-19 vaccines on humans
- Placebo controlled clinical trials
- Published in peer reviewed journals
- Published between 1 January 2019 and 12 March 2021

2.2.1 PubMed, Cochrane Central Register of Controlled Trials (EBSCO) and Medline ProQuest literature search

The search options for all databases were set to include only clinical trials or randomized clinical trials available in free full text or full text and published in English language between 1 January 2019 and 12 March 2021. Additional filters were added to include only human subjects and to exclude review articles when possible. Publications were excluded if they did not meet the inclusion criteria.

The search from PubMed resulted in 27 publications from which 14 publications met the inclusion criteria. Thirteen results were excluded. The search from Cochrane Central Register of Controlled Trials (EBSCO) resulted in 6 publications from which 3 met the inclusion criteria and were duplicates. Three results were excluded. The search from Medline ProQuest database resulted in 20 publications from which 0 met the inclusion criteria. All results were excluded.

2.3. Review of COVID-19 vaccines in the European Union

This part of the study aimed to review characteristics of the COVID-19 vaccines which are either authorized for use in the European Union or under rolling review by the European Medicines Agency (EMA) in 12 March 2021. The official website of the EMA was used to identify these vaccines. The search resulted in 4 vaccines which are available via conditional marketing authorization and 3 vaccines which are under rolling review.

2.4. Ethics approval

Ethics approval for conducting this study was granted from The University of Malta Research and Ethics Committee (UREC) (Appendix 1).

CHAPTER 3

RESULTS

3.1. Clinical trials on safety and efficacy of COVID-19 vaccines

The publications that were obtained via the literature search are listed in Table 3.1 and numbered in chronological order according to publication date from oldest to newest. Two of the publications included an interim analysis of more than one clinical trial. Number 1 included 2 trials (Phase 1 and 2) and number 10 included four trials (COV001, COV002, COV003 and COV005). COV001 and COV002 are included as separate publications (numbers 3 and 7).

Four out of 14 of the studies included novel mRNA vaccines (Table 3.2). Three studies examined inactivated SARS-CoV-2 vaccines. Three studies included a chimpanzee adenovirus-vectored vaccine. One study included a recombinant adenovirus type-5-vectored COVID-19 vaccine. SARS-CoV-2 recombinant spike protein nanoparticle vaccine was examined in one study. One study included a protein subunit vaccine and another study evaluated a recombinant adenovirus vaccine.

All of the studies were interventional placebo-controlled trials and all were blinded. Five of the studies were phase 1/2 studies, 2 were phase 1 studies, 2 were phase 2/3 studies, 2 were phase 3 studies and 1 was a phase 2 study. One study included two separate phase 1 and 2 studies. One study included four trials with phases ranging from phase 1/2 to phase 3. Four studies were conducted in China. Two studies were conducted in United States. Two studies were conducted in the United Kingdom and 2 in Australia. One study was done in Germany and 1 in Russia. Two studies were conducted in multiple sites. One study included the United States, Argentina, Brazil, South Africa, Germany, Turkey and the other study included the United Kingdom, Brazil and South Africa.

Table 3.1: Publications on COVID-19 vaccine clinical trials

Number	Name	Reference
1.	Effect of an Inactivated Vaccine Against SARS-CoV-2 on Safety and Immunogenicity Outcomes: Interim Analysis of 2 Randomized Clinical Trials	Xia et al, 2020
2.	Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebocontrolled, phase 2 trial	Zhu et al, 2020
3.	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	Folegatti et al, 2020
4.	Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults	Mulligan et al, 2020
5.	Phase 1–2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine	Keech et al, 2020
6.	Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates	Walsh, et al 2020
7.	Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial	Ramasamy et al, 2020
8.	Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine	Polack et al, 2020
9.	Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial	Xia et al, 2021
10.	Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK	Voysey et al, 2021
11.	Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial	Zhang et al, 2021
12.	Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine	Baden et al, 2021
13.	Safety and immunogenicity of S-Trimer (SCB-2019), a protein subunit vaccine candidate for COVID-19 in healthy adults: a phase 1, randomised, double-blind, placebo-controlled trial	Richmond et al, 2021
14.	Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia	Logunov et al, 2021

Table 3.2: Published clinical trials on safety and efficacy of COVID-19 vaccines

Number	Vaccine type(s)	Study type	Study phase	Study location	Reference
1.	Inactivated SARS-Cov-2 vaccine	Interventional, placebo-controlled, double-blinded	Phase 1 and phase 2	China	Xia et al, 2020
2.	Recombinant adenovirus type-5-vectored COVID-19 vaccine	Interventional, placebo-controlled, double-blinded	Phase 2	China	Zhu et al, 2020
3.	Chimpanzee adenovirus-vectored vaccine	Interventional, placebo-controlled, single-blinded	Phase 1/2	United Kingdom	Folegatti et al, 2020
4.	mRNA vaccine	Interventional, placebo-controlled, observer-blinded	Phase 1/2	Germany	Mulligan et al, 2020
5.	SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine	Interventional, placebo-controlled, observer-blinded	Phase 1/2	Australia	Keech et al, 2020
6.	mRNA vaccines	Interventional, placebo-controlled, observer blinded	Phase 1	United States	Walsh, et al 2020
7.	Chimpanzee adenovirus-vectored vaccine	Interventional, placebo-controlled, single-blinded	Phase 2/3	United Kingdom	Ramasamy et al, 2020
8.	mRNA vaccine	Interventional, placebo-controlled, observer-blinded	Phase 2/3	United States, Argentina, Brazil, South Africa, Germany, Turkey	Polack et al, 2020
9.	Inactivated SARS-CoV-2 vaccine	Interventional, placebo-controlled, double-blinded	Phase 1/2	China	Xia et al, 2021

Number	Vaccine type(s)	Study type	Study phase	Study location	Reference
10.	Chimpanzee adenovirus-vectored vaccine	Interventional, placebo-controlled COV003: single blinded COV005: double-blinded	COV003: Phase 3 COV005: Phase 1/2	COV003: Brazil COV005: South Africa	Voysey et al, 2021
11.	Inactivated SARS-CoV-2 vaccine	Interventional, placebo-controlled, double-blinded	Phase 1/2	China	Zhang et al, 2021
12.	mRNA vaccine	Interventional, placebo-controlled, observer-blinded	Phase 3	United States	Baden et al, 2021
13.	Protein subunit vaccine	Interventional, placebo-controlled, double-blinded	Phase 1	Australia	Richmond et al, 2021
14.	Recombinant adenovirus vaccine	Interventional, placebo-controlled, double-blinded	Phase 3	Russia	Logunov et al, 2021

3.2. Clinical trials participants' demographics

Thirteen studies included male and female adults over the age of 18 (Table 3.3). Only one study included participants aged between 16 – 18 years. None of the studies included children. Previous COVID-19 or exposure to SARS-CoV-2 was a common exclusion criterion. In three studies, sufficient risk of being exposed to SARS-CoV-2 during the study was considered as an inclusion criterion. Serious illnesses such as HIV and hepatitis B and C, were commonly considered as exclusion criteria. However, three studies included HIV positive participants. Women who were pregnant or breastfeeding were excluded in all studies. The races or ethnicities of the participants in the studies included Caucasians, Asians, Blacks, American Indians, Pacific Islanders, Hispanics and multiracial people.

Table 3.3: Clinical trials participants demographics

Study number	Main eligibility criteria	Reference
1.	Healthy adults, between 18 to 59 years-old with no history of SARS-CoV-1 or SARS-CoV-2 who were committed to completing the study and had the capability to understand the procedures and give consent	Xia et al, 2020
2.	Healthy adults over the age of 18, who were committed to completing the study with body temperature less or equal to 37.0°C and BMI within 18.5 - 30.0. People with HIV, psychiatric disease, allergies, serious cardiovascular disease, other serious disease, pregnancy or breastfeeding and confirmed previous SARS-CoV-2 were excluded	Zhu et al, 2020
3.	Adults 18 to 55 years old with no laboratory confirmed history of previous COVID-19	Folegatti et al, 2020
4.	Adults 18–55 years of age. People with HIV, hepatitis B and C and previous COVID-19 were excluded	Mulligan et al, 2020
5.	Healthy adults between 18 to 59 years of age with a BMI withing the range of 17 to 35. People with history of SARS-CoV-2 infection and being at high risk of SARS-CoV-2 exposure were excluded	Keech et al, 2020
6.	Healthy adults who were either 18 - 55 years old and 65 - 85 years old. People with HIV, Hepatitis C or B, autoimmune diseases, previous COVID-19 or previous vaccination against- or medication to prevent COVID-19 were excluded	Walsh, et al 2020
7.	Healthy adults who were either 18-55 years, 56-69 years and people over 70 years of age. Exclusion criteria were severe or uncontrollable co-morbidities and Dalhousie Clinical Frailty Score of 4	Ramasamy et al, 2020
8.	People 16 years of age or older who are healthy or have a stable chronic medical condition (including HIV). Participants with previous COVID-19, immunosuppressive treatment or immunocompromising condition were excluded	Polack et al, 2020
9.	Healthy adults 18–80 years-old with no previous infection of SARS-CoV-2 and who were able to comply with the schedule. People with history of travelling to Hubei province, outside of China or areas with known COVID-19 cases from December 2019, mental illness, pregnancy or breastfeeding, laboratory abnormalities, allergies to the vaccine ingredients, history of seizures were excluded	Xia et al, 2021
10.	COV003: Adults over the age of 18 with high risk of exposure to the SARS-COV-2. People with prior COVID-19, HIV,	Voysey et al, 2021

Study number	Main eligibility criteria	Reference
	history of anaphylaxis and pregnant or breastfeeding women were excluded COV005: Adults 18 to 65 years-old including HIV positive people. People with prior COVID-19, history of anaphylaxis and pregnant or breastfeeding women were excluded	
11.	Healthy adults 18 to 59 years-old. People with prior SARS-CoV-2, history of being in contact with a person with someone infected with SARS-CoV-2, fever, allergy to vaccine ingredients and travel history to Wuhan city were excluded	Zhang et al, 2021
12.	People 18 years of age or older (including HIV positive people) who were at reasonable risk for SARS-CoV-2 infection. People with previous history of SARS-CoV-2 infection were excluded	Baden et al, 2021
13.	Healthy adults 18–75 years of age who had a BMI between 18.5–35.0 and were able to understand and sign consent. People with previous SARS-CoV-2, uncontrolled medical conditions, allergy to any vaccine, malignant diseases such as HIV, Hepatitis B and C, previous COVID-19 vaccination, immune system impairment and women who were either pregnant or breastfeeding were excluded	Richmond et al, 2021
14.	People 18 years or older. People with previous COVID-19, HIV, hepatitis B and C, syphilis and pregnant women were excluded	Logunov et al, 2021

3.3. Study designs

The included studies implemented various study group designs (Table 3.4). The designs included dividing participants into different age groups, vaccine dose groups, different dosing days groups and adjuvanted or non-adjuvanted groups. One study included two different vaccines and the participants received either one of the vaccines or a placebo. All the studies included control groups who were administered active or passive placebo injections. Study number 10 differed from the other studies in that they included the use of paracetamol in the COV003 trial.

Table 3.4: Study Groups

Study number	Study groups	Reference
1.	**Phase 1** **Group 1:** 2.5 mcg dose of vaccine, n=24 **Group 2:** 5.0 mcg of vaccine, n=24 **Group 3:** 10 mcg of vaccine, n=24 **Group 4**: Placebo, n=24 **Phase 2** **Group 1:** 5.0 mcg of vaccine on days 0 and 14, n=84 **Group 2:** Placebo on days 0 and 14, n=28 **Group 3:** 5.0 mcg of vaccine on days 0 and 21, n=84 **Group 4**: Placebo on days 0 and 21, n=28	Xia et al, 2020
2.	**Group 1:** 1×10^{11} viral particles (vp) dose of vaccine, n=253 **Group 2**: 5×10^{10} vp dose of vaccine, n=129 **Group 3**: Placebo, n=126	Zhu et al, 2020
3.	**Group 1:** 5×10^{10} vp single dose of vaccine, n=44 vs placebo, n=44 (had intensive early follow-ups for safety and immunogenicity) **Group 2:** 5×10^{10} vp single dose of vaccine, n=489 vs placebo, n=490 **Group 3:** 5×10^{10} vp dose of vaccine on days 0 and 28, n=10 Group 2 was further divided into two groups where higher blood volumes were taken for immunogenicity assessment in the other group.	Folegatti et al, 2020
4.	**Group 1:** 10 mcg dose of vaccine, n=12 vs. placebo, n=3 **Group 2:** 30 mcg dose of vaccine, n=12 vs. placebo, n=3	Mulligan et al, 2020

Study number	Study groups	Reference
	Group 3: 100 mcg dose of vaccine, n=12 vs. placebo, n=3	
5.	**Group 1:** Placebo, n=26 **Group 2:** 25 mcg dose of vaccine on days 0 and 21, n=25 **Group 3:** 5 mcg dose of vaccine on days 0 and 21 with adjuvant, n=25 **Group 4:** 25 mcg dose of vaccine on days 0 and 21 with adjuvant, n=25 **Group 5:** 25 mcg dose of vaccine on day 0 with adjuvant, n=25	Keech et al, 2020
6.	**Group 1:** BNT162b1 (18–55yr), n=60 **Group 2:** BNT162b1 (65–85yr), n=45 **Group 3:** BNT162b2 (18–55yr), n=45 **Group 4**: BNT162b2 (65–85yr), n=45 Each group was further divided into either, 10 mcg, 20 mcg, 30 mcg or placebo. The BNT162b1 (18–55yr) group included also a 100 mcg dose.	Walsh, et al 2020
7.	**Group 1:** 2.2 x 10^{10} vp dose of vaccine on days 0 and 28 (18-55yr), n=50 vs placebo, n=49 **Group 2:** 2.2 x 10^{10} vp dose of vaccine on day 0 (56-69yr), n=30 vs placebo, n=10 **Group 3:** 2.2 x 10^{10} vp dose of vaccine on days 0 and 28 (56-69yr), n=30 vs placebo, n=10 **Group 4:** 2.2 x 10^{10} vp dose of vaccine on day 0 (70+yr), n=50 vs placebo, n=10 **Group 5:** 2.2 x 10^{10} vp dose of vaccine on days 0 and 28 (70+yr), n=46 vs placebo, n=10 **Group 6:** 3.5 - 6.5 x 10^{10} vp dose of vaccine on days 0 and 28 (18-55yr), n=49 vs placebo, n=9 **Group 7:** 3.5 - 6.5 x 10^{10} vp dose of vaccine on day 0 (56-69yr), n=30 vs placebo, n=10	Ramasamy et al, 2020

Study number	Study groups	Reference
	Group 8: 3.5 - 6.5 x 10^{10} vp dose of vaccine on days 0 and 28 (56-69yr), n=30 vs placebo, n=10 **Group 9:** 3.5 - 6.5 x 10^{10} vp dose of vaccine on day 0 (70+yr), n=50 vs placebo, n=10 **Group 10:** 3.5 - 6.5 x 10^{10} vp dose of vaccine on days 0 and 28 (70+yr), n=49 vs placebo, n=10	
8.	**Group 1:** 30 mcg dose of vaccine on days 0 and 21, n=18,556 **Group 2:** placebo on days 0 and 21, n=18,530	Polack et al, 2020
9.	**Phase 1** **Group 1:** 2 mcg dose of vaccine on days 0 and 28 (18-59yr), n=24 vs placebo, n=8 **Group 2:** 4 mcg dose of vaccine on days 0 and 28 (18-59yr), n=24 vs placebo, n=8 **Group 3:** 8 mcg dose of vaccine on days 0 and 28 (18-59yr), n=24 vs placebo, n=8 **Group 4:** 2 mcg dose of vaccine on days 0 and 28 (60+yr), n=23 vs placebo, n=8 **Group 5:** 4 mcg dose of vaccine on days 0 and 28 (60+yr), n=24 vs placebo, n=8 **Group 6:** 8 mcg dose of vaccine on days 0 and 28 (60+yr), n=23 vs placebo, n=7 **Phase 2** **Group 1:** 8 mcg single dose of vaccine, n=84 vs placebo, n=28 **Group 2:** 4 mcg dose of vaccine on days 0 and 14, n=84 vs placebo, n=28 **Group 3:** 4 mcg dose of vaccine on days 0 and 21, n=84 vs placebo, n=28 **Group 4:** 4 mcg dose of vaccine on days 0 and 28, n=84 vs placebo, n=28	Xia et al, 2021

Study number	Study groups	Reference
10.	**COV003** **Group 1:** A single 3.5 – 6.5 × 10^{10} vp dose of vaccine, n= up to 1600 and paracetamol vs placebo and paracetamol, n= up to 1600 **Group 2:** Two 3.5 – 6.5 × 10^{10} vp doses of vaccine separated by 4 to 12 weeks and paracetamol, n= up to 5150 vs two doses of placebo separated by 4 to 12 weeks and paracetamol, n= up to 5150 **COV005** **Group 1:** Two 5-7.5x10^{10} vp doses of vaccine separated by 4 weeks with intensive follow up vs placebo, total n=70 **Group 2:** Two 5-7.5x10^{10} vp doses of vaccine separated by 4 weeks with intensive immunogenicity assessment vs placebo, total n=250 **Group 3:** Two 5-7.5x10^{10} vp doses of vaccine separated by 4 weeks with safety and efficacy assessments vs placebo, total n=1650 **Group 4:** Two 5-7.5x10^{10} vp doses of vaccine separated by 4 weeks with prime boost vs placebo in HIV positive participants, total n=100	Voysey et al, 2021
11.	**Phase 1** **Group 1:** 3 mcg dose of vaccine on days 0 and 14, n=24 vs placebo, n=12 **Group 2:** 6 mcg dose of vaccine on days 0 and 14, n=24 vs placebo, n=12 **Group 3:** 3 mcg dose of vaccine on days 0 and 28, n=24 vs placebo, n=12 **Group 4:** 6 mcg dose of vaccine on days 0 and 28, n=24 vs placebo, n=11 **Phase 2** **Group 1:** 6 mcg dose of vaccine on days 0 and 14, n=119 **Group 2:** 3 mcg dose of vaccine on days 0 and 14, n=120 **Group 3:** Placebo on days 0 and 14, n=60	Zhang et al, 2021

Study number	Study groups	Reference
	Group 4: 6 mcg dose of vaccine on days 0 and 28, n=118 **Group 5:** 3 mcg dose of vaccine on days 0 and 28, n=117 **Group 6:** Placebo on days 0 and 28, n=60	
12.	**Group 1:** 100 mcg of vaccine on days 0 and 28, n=15,181 **Group 2:** placebo on days 0 and 28, n=15,170	Baden et al, 2021
13.	**Group 1:** 3 mcg dose of vaccine on days 0 and 21, n=8 **Group 2:** 3 mcg dose of vaccine with AS03 adjuvant on days 0 and 21, n=16 **Group 3:** 3 mcg dose of vaccine with CpG/Alum adjuvant on days 0 and 21, n=16 **Group 4:** 9 mcg dose of vaccine on days 0 and 21, n=8 **Group 5:** 9 mcg dose of vaccine with AS03 adjuvant on days 0 and 21, n=16 **Group 6:** 9 mcg dose of vaccine with CpG/Alum adjuvant on days 0 and 21, n=15 **Group 7:** 30 mcg dose of vaccine on days 0 and 21, n=7 **Group 8:** 30 mcg dose of vaccine with AS03 adjuvant on days 0 and 21, n=16 **Group 9:** 30 mcg dose of vaccine with CpG/Alum adjuvant on days 0 and 21, n=16 **Group 10:** placebo on days 0 and 21, n=30	Richmond et al, 2021
14.	**Group 1:** 10^{11} vp dose of vaccine on days 0 and 21, n=14,964 **Group 2:** placebo on days 0 and 21, n=4902	Logunov et al, 2021

The efficacy assessments varied between the included studies (Table 3.5). Eleven studies included neutralizing antibodies against SARS-CoV-2 as an immunogenicity assessment. Immunoglobulin G (IgG) against the receptor binding domain (RBD) and the spike protein as well as T-Cell responses were also commonly measured. Four studies assessed the efficacy of the vaccines against COVID-19. One of these studies assessed both the immunologic responses and the efficacy against COVID-19. Safety assessments in the studies included solicited and unsolicited local and systemic adverse reactions as well as laboratory abnormalities. Three studies included also the use of antipyretic medications as a safety assessment.

Table 3.5: Main efficacy and safety assessments

Study number	Main efficacy and immunogenicity assessment(s)	Main safety assessment(s)	Reference
1.	Neutralizing antibody responses against SARS-CoV-2 and specific IgG-binding antibody responses	Local and systemic adverse reactions	Xia et al, 2020
2.	Neutralizing antibody responses to live SARS-CoV-2 virus and pseudovirus, specific antibody responses against the RBD and T-Cell responses	Local and systemic adverse reactions	Zhu et al, 2020
3.	Neutralizing antibodies against SARS-CoV-2, IgGs against spike protein and antigen-specific T-Cell responses	Local and systemic adverse reactions	Folegatti et al, 2020
4.	Neutralizing antibody responses against SARS-CoV-2 and RBD-binding IgGs	Local and systemic adverse reactions and use of antipyretic or pain medication after the vaccination	Mulligan et al, 2020
5.	Neutralizing antibodies against SARS-CoV-2, IgG responses against the spike protein and T-Cell responses	Local and systemic adverse reactions	Keech et al, 2020
6.	Neutralizing antibody responses against SARS-CoV-2, RBD-binding or spike protein-binding IgGs	Local and systemic adverse reactions and use of antipyretic or	Walsh, et al 2020

Study number	Main efficacy and immunogenicity assessment(s)	Main safety assessment(s)	Reference
		pain medication after the vaccination	
7.	Neutralizing antibody responses against SARS-CoV-2, RBD-binding IgG responses and IgGs against spike protein, antigen-specific T-Cell responses	Local and systemic adverse reactions	Ramasamy et al, 2020
8.	The efficacy of BNT162b2 vaccine against COVID-19	Local and systemic adverse events and the use of antipyretic medication	Polack et al, 2020
9.	Neutralizing antibodies against SARS-CoV-2	Local and systemic adverse reactions	Xia et al, 2021
10.	The efficacy of ChAdOx1 nCoV-19 vaccine against COVID-19 from four trials: COV001 (number 3), COV002 (number 7), COV003 and COV005	Local and systemic adverse reactions	Voysey et al, 2021
11.	Neutralizing antibodies against SARS-CoV-2, RBD-specific IgGs, spike protein specific IgGs and IgMs, T-Cell responses and neutralizing antibodies against pseudovirus	Local and systemic adverse reactions, changes in laboratory values or serum inflammatory factors	Zhang et al, 2021
12.	The efficacy of mRNA-1273 vaccine against COVID-19	Local and systemic adverse reactions	Baden et al, 2021
13.	Neutralizing antibodies against SARS-CoV-2, IgG antibodies against SCB-2019, ACE2-competitive blocking IgG antibodies and T-Cell responses against pooled S-protein	Local and systemic adverse reactions	Richmond et al, 2021
14.	The efficacy of rAd26 and rAd5 vector-based vaccine against COVID-19. Antibody responses against SARS-CoV-2 glycoprotein S and SARS-CoV-2 N-protein, neutralizing antibodies against SARS-CoV-2 and cellular responses were assessed	The incidence and severity of adverse reactions	Logunov et al, 2021

3.4. Main adverse effects resulted from the studies

Various adverse reactions resulting from vaccination or placebo were reported in the included studies (Appendix 2). Pain in some form was reported as an adverse reaction in all the included studies where data was available. Common local adverse reactions were swelling, redness, itch and tenderness. Fever was reported as a systemic adverse reaction in all the included studies where data was available. Other common systemic adverse reactions were fatigue, headache, chills, diarrhea, nausea and malaise. Two studies reported possibly vaccine related serious adverse reactions. These serious adverse reactions included transverse myelitis, fever higher than 40°C, Shoulder injury from injection, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia and right leg paresthesia (studies 8 and 10).

3.5. Main efficacy results

Efficacy was reported with either immunologic responses to vaccination or as efficacy against COVID-19 disease (Table 3.6). Eleven studies that assessed immunologic responses resulting from vaccination showed neutralizing antibody responses against SARS-CoV-2. The vaccines commonly elicited responses in IgGs as well as T-Cell mediated immunity. Four of the studies assessed the vaccine efficacy against COVID-19. All the vaccines in these four studies elicited higher than 70% efficacy against COVID-19 ranging from 70.4% to 95%.

Table 3.6: Main efficacy results

Number	Main efficacy findings	Reference
1.	The inactivated vaccine elicited immunologic responses in neutralizing antibodies against SARS-CoV-2 and in the specific IgG antibodies	Xia et al, 2020
2.	The Ad5-vectored COVID-19 vaccine elicited immunologic responses with both vaccine doses in neutralizing antibodies against SARS-CoV-2, RBD antibodies, T-Cells and in pseudovirus neutralizing antibodies	Zhu et al, 2020
3.	The ChAdOx1 nCoV-19 vaccine elicited immunologic responses in neutralizing antibodies against SARS-CoV-2, IgGs against spike protein and in T-Cells	Folegatti et al, 2020
4.	The BNT162b1 vaccine elicited immunologic responses in neutralizing antibodies against SARS-CoV-2 and in the RBD-binding IgG antibodies	Mulligan et al, 2020
5.	The SARS-CoV-2 recombinant spike protein nanoparticle vaccine elicited immunologic responses in neutralizing antibodies against SARS-CoV-2, IgGs against spike protein and in T-Cells	Keech et al, 2020
6.	BNT162b1 and BNT162b2 vaccines both elicited responses in neutralizing antibodies against SARS-CoV-2 and in antigen specific IgGs	Walsh, et al 2020
7.	The ChAdOx1 nCoV-19 vaccine elicited immunologic responses in neutralizing antibodies against SARS-CoV-2, IgGs against spike protein and in T-Cells	Ramasamy et al, 2020
8.	The BNT162b2 vaccine elicited 95% efficacy against COVID-19 with 95% confidence interval (90.3-97.6)	Polack et al, 2020
9.	The BBIBP-CorV vaccine elicited neutralizing antibodies against SARS-CoV-2	Xia et al, 2021
10.	In combining preliminary results from four different trials COV001 (number 3), COV002 (number 7), COV003 and COV005 the ChAdOx1 nCoV-19 showed 70.4% efficacy against COVID-19	Voysey et al, 2021
11.	The CoronaVac vaccine elicited neutralizing antibodies against SARS-CoV-2	Zhang et al, 2021
12.	The mRNA-1273 vaccine elicited 94.1% efficacy against COVID-19 with 95% confidence interval (89.3 - 96.8).	Baden et al, 2021
13.	The adjuvanted (AS03 or CpG/Alum) SCB-2019 vaccine elicited responses in neutralizing antibodies, IgGs and T-Cells	Richmond et al, 2021
14.	The rAd26 and rAd5 vector-based vaccine elicited 91.6% efficacy against COVID-19 with 95% confidence interval (85.6–95.2) as well as responses in SARS-CoV-2 neutralizing antibodies and in RBD-specific IgGs	Logunov et al, 2021

3.6. COVID-19 vaccines authorized for use

The COVID-19 vaccines that were authorized for use in the European Union by 12 March 2021 utilize various vaccine technologies and includes the new mRNA vaccines, chimpanzee adenovirus vaccine and a non-replicating viral vector vaccine.[13] The first COVID-19 vaccine that was granted a conditional marketing authorization after evaluation by the human medicines committee (CHMP) in the European Union was the Pfizer and BioNTech's Comirnaty (BNT162b2) mRNA vaccine in 21 December 2020.[23] This decision was based on a large clinical trial that included around 44,000 people which showed 95% efficacy against COVID-19. This was followed by the second conditional marketing authorization for the Moderna's mRNA-1273 COVID-19 vaccine which was granted on 6 January 2021.[24] The decision was taken after clinical trial including around 30,000 people showing 94.1% efficacy against COVID-19. In 29 January 2021 the European Medicines Agency (EMA) granted a conditional marketing authorization to the AstraZeneca's AZD1222 COVID-19 vaccine based on four clinical trials including a total of around 24,000 people that showed 59.5% efficacy against COVID-19.[25] The fourth COVID-19 vaccine that has been granted a conditional marketing authorization on 11 March 2021 is the Ad26.COV2.S also known as COVID-19 Vaccine Janssen.[26] The approval of the COVID-19 Vaccine Janssen was based

[23] European Medicines Agency (EMA). EMA recommends first COVID-19 vaccine for authorisation in the EU [Internet]. EMA; 2020 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-recommends-first-covid-19-vaccine-authorisation-eu

[24] European Medicines Agency (EMA). EMA recommends COVID-19 Vaccine Moderna for authorisation in the EU [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-moderna-authorisation-eu

[25] European Medicines Agency (EMA). EMA recommends COVID-19 Vaccine AstraZeneca for authorisation in the EU [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-astrazeneca-authorisation-eu

[26]European Medicines Agency (EMA). EMA recommends COVID-19 Vaccine Janssen for authorisation in the EU [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-janssen-authorisation-eu

on a large multinational clinical trial including more than 44,000 people that showed a 67% efficacy against COVID-19.

3.6.1. Pfizer and BioNTech: Comirnaty (BNT162b2)

The BNT162b2 developed by Pfizer and BioNTech is a nucleoside-modified RNA vaccine coated with a lipid nanoparticle layer and it encodes the full SARS-CoV-2 spike protein including two proline modifications (Polack et al, 2020). The mRNA is manufactured using fermented *Escherichia coli* cell template with adenosine triphosphate, cytidine triphosphate, guanosine triphosphate, modified uridine triphosphate and the 5' Cap (MHRA, 2020). The proline mutations in the mRNA are located in the central helix which results in the spike protein to be in the antigenically preferable conformation.[27]

The Comirnaty vaccine includes two novel lipids ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)) (ALC-0315) and (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide) (ALC-0159) in addition to the two already approved cholesterol and 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) (EMA, 2021a). The ALC-0315 is a cationic lipid that in combination with nanoparticles is used to regulate the intracellular release of the RNA (MHRA, 2020). The ALC-0315 interacts with the RNA in a pH dependent manner so that specific pH connects the RNA with the lipid and the lower pH inside the cell results in the intracellular release of the RNA. The second novel hydrophilic lipid component, the ALC-0159, serves the purpose of protecting the lipid nano particles

[27] BioNTech. Comirnaty: EPAR - Product Information [Internet]. European Medicines Agency. Last updated: February 25, 2021 [cited 2021 Mar 27]. Available from URL: https://www.ema.europa.eu/en/medicines/human/EPAR/comirnaty#product-information-section

which improves their stability (MHRA, 2020). The cholesterol and DSPC are included to stabilize and support the bilayer structures of the lipids (MHRA, 2020).

The Comirnaty vaccine in its final product form is a sterile dispersion of the mRNA protected by the four lipids with a cryoprotectant buffer in a pH of 6.9 – 7.9 (EMA, 2021a). The dispersion is stored in 0.45 ml vials each containing six 0.3 ml doses after it is diluted.[27] Each 0.3 ml dose contains 30 mcg of mRNA. The Comirnaty vaccine requires a very low temperature of around −80°C for storage (Poland et al, 2020). To mitigate the challenge of supplying the vaccines around the world while maintaining the integrity of the product, Pfizer has developed specialized packaging solutions for distributing the vaccines that keep the low temperatures for up to 10 days.[28] The vaccines can then be stored in ultra-low-temperature freezers for 6 months. The storage unit developed by Pfizer can be also refilled with dry ice is which allows for additional 30 days of storage. The BNT162b2 vaccine can be stored in normal refrigeration temperatures for five days.[28]

When prepared for administration, the preparation is diluted in the vial with 1.8 ml of sodium chloride 9 mg/ml solution for injection, after which it needs to be gently inverted about ten times to produce a homogenous dispersion.[27] The vaccine is preferably administered to the deltoid muscle with two doses separated by 21 days (MHRA, 2020). Intravascular, subcutaneous and intradermal injection are prohibited.[27]

The pharmacokinetic studies done in rats showed that intramuscular injection with modified mRNA and nanoparticles similar to the Comirnaty vaccine, results in peak concentrations in the protein expression after 6h (EMA, 2021a). These results were observed most prominently at the injection site and in liver but other tissues are affected as well. The mRNA expression

[28] Pfizer. COVID-19 Vaccine U.S. Distribution Fact Sheet [Internet]. [cited 2021 Feb 16]. Available from URL: https://www.pfizer.com/news/hot-topics/covid_19_vaccine_u_s_distribution_fact_sheet

returned to baseline levels after 9 days at the injection site and after 48h in the liver area (EMA, 2021a). The studies on the novel lipid compounds showed elimination half-lives to be 2-3 days for the ALC-0159 and 6-8 days for the ALC0315 (MHRA, 2020). The same lipid structure that is used in the Comirnaty vaccines has been shown to elicit increased levels of MCP-1, IL-6, IP-10 as well as induce an IFN-γ T-Cell response in rats (EMA, 2021a). The pharmacodynamics of the Comirnaty vaccine have been demonstrated in a placebo-controlled phase 1 trial with human subjects (Walsh et al, 2020). Comirnaty vaccination demonstrated dose depended immunologic responses measured by SARS-CoV-2 neutralizing antibodies and antigen specific IgGs. The Comirnaty vaccine has demonstrated 95% protection against COVID-19 in a placebo-controlled clinical trial (Polack et al, 2020).

3.6.2. Moderna: mRNA-1273

Another mRNA COVID-19 vaccine is the mRNA-1273 developed by Moderna that includes a mRNA sequence and a lipid nanoparticle component (EMA, 2021b). The mRNA-1273 encodes the S-2P antigen that consists of the SARS-CoV-2 spike protein (Jackson et al, 2020). This glycoprotein includes the transmembrane anchor and an intact S1–S2 cleavage site. The researchers stabilized the S-2P to its prefusion conformation by substituting amino acids with proline in the positions 986 and 987 that are found in the top of the central helix in the S2 subunit (Jackson et al, 2020). The mRNA component of the vaccine uses a DNA template from *Escherichia coli* cell line and includes a 5’ cap, the 5’ untranslated region, the Open Reading Frame, the 3’ untranslated region, and 3’ polyA tail (EMA, 2021b).

The mRNA sequence in the vaccine is surrounded and protected by a lipid component consisting of 4 different lipids: SM-102, PEG2000-DMG, cholesterol and DSPC (MHRA, 2021a). The SM-102 is a novel lipid which interacts with the mRNA due to its positive

charge, and the other novel lipid PEG2000-DMG improves the steric stability of the structure (EMA, 2021b). The cholesterol is used for structural support and the DSPC is included to improve the cellular intake of the mRNA (MHRA, 2021a).

The Moderna COVID-19 vaccine product is a sterile, white to off white dispersion with pH between 7.0 and 8.0.[29] The vaccine is stored in temperatures of -25ºC to -15ºC and when stored in 2° to 8°C temperatures, it can be used within 30 days.[30] The vaccine is supplied in preservative-free type 1 glass vials each containing 6.3 ml of the vaccine (MHRA, 2021a). Each vial contains enough vaccine for 10 doses. The single 0.5 ml dose contains 100 mcg of the mRNA (EMA, 2021b). The Moderna COVID-19 vaccine does not require dilution prior to use and is preferably administered to the deltoid muscle in the arm in two doses separated by 28 days.[29] The product should be thawed prior to use and should not be given intravascularly, subcutaneously or intradermally.[29]

Pharmacokinetic properties of the lipid nanoparticles used in the Moderna mRNA vaccine have been studied in rats using a different mRNA component (EMA, 2021b). The lipids used in this study were different size than in the actual Moderna COVID-19 vaccine and the possible impact of this to its pharmacokinetic properties is not clear. The vaccine (mRNA-1647) was given intramuscularly to rats with a dose of 100 mcg (MHRA, 2021a). The peak concentration of the mRNA products was detected after 2 hours from administration in multiple tissues and plasma. The half-life of the mRNA-1647 is estimated to be around 2.7 - 3.8 hours (EMA, 2021b). The pharmacodynamic properties of the Moderna COVID-19 vaccine has been demonstrated in human subjects (Jackson et al, 2020). This study assessed

[29] Moderna. COVID-19 Vaccine Moderna: EPAR - Product information [Internet]. European Medicines Agency. Last updated: March 23, 2021 [cited 2021 Mar 27]. Available from URL: https://www.ema.europa.eu/en/medicines/human/EPAR/covid-19-vaccine-moderna
[30] Moderna. Storage & Handling: Moderna COVID-19 Vaccine (EUA) [Internet]. [cited 2021 Feb 16]. Available from URL: https://www.modernatx.com/covid19vaccine-eua/providers/storage-handling

the immunologic responses of two dose mRNA-1273 regimen with SARS-CoV-2 specific antibodies, neutralizing antibodies and T-Cell responses. All participants showed immunologic responses against SARS-CoV-2 (Jackson et al, 2020).

3.6.3. AstraZeneca: AZD1222

The AZD1222 is a chimpanzee adenovirus vaccine developed together with AstraZeneca and the Oxford Vaccine Group at the University of Oxford (MHRA, 2021b). The AZD1222 is based on replication deficient chimpanzee adenoviral vector that includes the genomic information for coding the SARS-CoV-2 spike protein (Voysey et al, 2021). Adenoviruses are non-encapsulated virions with diameters ranging from 80 to 100 nm and their viral capsids comprise of three major proteins and four minor proteins (MHRA, 2021b). The manufacturing of the AZD1222 is done in T-REx-293 cells which are derived from human kidneys (EMA, 2021c). The SARS-CoV-2 spike protein component uses a modified human cytomegalovirus promoter as well as bovine growth hormone polyadenylation sequence as an expression cassette (MHRA, 2021b).

The final vaccine product also contains L-histidine, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80, ethanol, sucrose, sodium chloride, disodium edetate dihydrate as well as water (EMA, 2021c). The AZD1222 vaccine does not require highly specialized conditions for storage or transportation as normal refrigerator temperature (2-8°C) is sufficient for storing the vaccine for 6 months. The vaccine product is a colourless to somewhat opalescent suspension (MHRA, 2021b). The vaccine vials have a rubber stopper and aluminum overseal with a plastic cap and they are shipped in 5 ml or 4 ml sizes containing 10 and 8 doses each respectively (MHRA, 2021b). A single 0.5 ml dose of the AZD1222 contains 5×10^{10} of the chimpanzee adenovirus-vectored vaccine coding

the spike protein (ChAdOx1-S) viral particles (WHO, 2021). The vaccine is intended to be administered only intramuscularly to the deltoid muscle of the upper arm as a two-injection regimen separated by 4 – 12 weeks.[31]

The AZD1222 is made replication deficient by deleting E1 regions of the genes and because of this, it is not expected that infections would occur outside the initial injection site (EMA, 2021c). Pharmacokinetic data from rat studies also suggest that when using similar viruses to the ChAdOx1-S, the virus does not meaningfully distribute outside the injection site (MHRA, 2021b). The AZD1222 has been demonstrated to elicit immunologic responses in human subjects via SARS-CoV-2 neutralizing antibodies, antigen specific IgGs and T-cell responses (Folegatti et al, 2020; Ramasamy et al, 2020). Another study showed 70.4% efficacy against COVID-19 in humans (Voysey et al, 2021).

3.6.4. Janssen: Ad26.COV2.S

The COVID-19 Vaccine Janssen also known as Ad26.COV2.S is a replication deficient type 26 adenovirus vector that includes the gene for coding the stabilized SARS-CoV-2 spike protein variant (FDA, 2021). This strain is manufactured in a PER.C6 TetR Cell Line with amino acids and proteins and then purified with multiple steps (FDA, 2021). In addition to the active ingredients, the vaccine contains 2-hydroxypropyl-β-cyclodextrin, citric acid monohydrate, ethanol, hydrochloric acid, polysorbate-80, sodium chloride, sodium hydroxide, trisodium citrate dihydrate and water.[32] The final product is a sterile, colorless to slightly yellow, clear to very opalescent suspension and is shipped in multidose vials each

[31] AstraZeneca. Vaxzevria (previously COVID-19 Vaccine AstraZeneca) EPAR - Product information [Internet]. European Medicines Agency. Last updated: March 26, 2021 [cited 2021Mar27]. Available from URL: https://www.ema.europa.eu/en/medicines/human/EPAR/vaxzevria-previously-covid-19-vaccine-astrazeneca#product-information-section

containing 5 doses of the vaccine.[32] The vaccine is stored at 2°C to 8°C and a single 0.5 ml dose contains 5×10^{10} viral particles (FDA, 2021).

Prior to administration of the Ad26.COV2.S vaccine the vial has to be swirled gently for 10 seconds but not shaken.[33] After the preparation the vaccine is injected intramuscularly as a single dose regimen which results in short term expression of the spike protein.[32] The administration of the Ad26.COV2.S vaccine to human subjects produces immunologic responses against SARS-CoV-2 measured with neutralizing antibodies and T-Cell responses (Sadoff et al, 2021). The Ad26.COV2.S vaccine has a 67% protection against symptomatic COVD-19 (EMA, 2021d).

3.7. COVID-19 vaccines under rolling review

A rolling review is used by the EMA to speed up assessment process of a medications with good potential in a public health emergency.[34] At the data lock point, 12 March 2021, the EMA has 3 vaccine candidates against COVID-19 under rolling review.[35] The rolling review for the NVX-CoV2373 vaccine by Novavax CZ AS started on 3 February 2021.[36] CureVac

[32] Janssen. COVID-19 Vaccine Janssen: EPAR - Product information [Internet]. European Medicines Agency. 2021 [cited 2021Mar27]. Available from URL: https://www.ema.europa.eu/en/medicines/human/EPAR/covid-19-vaccine-janssen#product-information-section

[33] Janssen. Fact Sheet For Healthcare Providers Administering Vaccine [Internet]. 2021. [Cited 2021 Mar 14]. Available from URL: https://www.janssenlabels.com/emergency-use-authorization/Janssen+COVID-19+Vaccine-HCP-fact-sheet.pdf

[34] European Medicines Agency (EMA). EMA starts rolling review of the Sputnik V COVID-19 vaccine [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-starts-rolling-review-sputnik-v-covid-19-vaccine

[35] European Medicines Agency (EMA). COVID-19 vaccines: under evaluation [Internet]. [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronaviru s-disease-covid-19/treatments-vaccines/vaccines-covid-19/covid-19-vaccines-under-evaluation

[36] European Medicines Agency (EMA). EMA starts rolling review of Novavax's COVID-19 vaccine (NVX-CoV2373) [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-starts-rolling-review-novavaxs-covid-19-vaccine-nvx-cov2373

AG's CVnCoV vaccines rolling review started on 12 February 2021.[37] and the Sputnik V by Russia's Gamaleya National Centre of Epidemiology and Microbiology rolling review started on 4 March 2021.[34]

3.7.1. Novavax CZ AS: NVX-CoV2373

The NVX-CoV2373 is a SARS-CoV-2 recombinant spike protein nanoparticle vaccine that is built from the full-length spike protein and manufactured in a baculovirus *Spodoptera frugiperda* (Sf9) insect cell expression system (Tian et al, 2021). The recombinant SARS-CoV-2 (rSARS-CoV-2) has a mutated S1/S2 cleavage site from 682- RRAR-685 to 682-QQAQ-685 to increase its protease resistance (Keeck et al, 2020; Tian et al, 2021). The rSARS-CoV-2 binds to the ACE2 receptor with high affinity (Keeck et al, 2020). The NVX-CoV2373 has been shown to tolerate pH values ranging from 4 to 9, freezing and thawing cycles and higher temperatures up to 37 °C without losing its ACE2 affinity but not in oxidizing conditions (Tian et al, 2021). The NVX-CoV2373 vaccine includes a proprietary Matrix-M adjuvant which has two particles each with different saponin fractions A and C (Magnusson et al, 2018; Keeck et al, 2020). The Matrix-M adjuvant enhances the immune reaction by increasing the amount of antigen presenting cells at the injection site and in local lymph nodes.[38] The NVX-CoV2373 can be stored at 2°- 8°C temperatures and is supplied in 10-dose vials.

[37] European Medicines Agency (EMA). EMA starts rolling review of CureVac's COVID-19 vaccine (CVnCoV) [Internet]. EMA; 2021 [cited 2021 Mar 20]. Available from URL: https://www.ema.europa.eu/en/news/ema-starts-rolling-review-curevacs-covid-19-vaccine-cvncov

[38] Novavax. Novavax Confirms High Levels of Efficacy Against Original and Variant COVID-19 Strains in United Kingdom and South Africa Trials [Internet]. [cited 2021 Mar 20]. Available from URL: https://ir.novavax.com/news-releases/news-release-details/novavax-confirms-high-levels-efficacy-against-original-and-0

The NVX-CoV2373 has been demonstrated to elicit immunological responses both in mice and in humans (Keeck et al, 2020; Tian et al, 2021). In clinical trials the NVX-CoV2373 has showed 96% efficacy COVID-19 and 100% protection from the severe form of the disease.[38]

3.7.2. CureVac AG: CVnCoV

The CVnCoV from CureVac AG is a lipid nanoparticle surrounded mRNA vaccine that codes the full-length SARS-CoV-2 spike protein (Kremsner et al, 2020). The CVnCoV includes a 5′ cap structure, a GC-enriched open reading frame and 3′ UTR, polyA tail (Rauch et al, 2020) The spike protein has an unmodified S1/S2 cleavage site and transmembrane domain but has two proline mutations, K986P and V987P, that stabilizes its conformation (Kremsner et al, 2020; Rauch et al, 2020). The CVnCoV lipid nano particles used in animal studies consist of an ionizable amino lipid, phospholipid, cholesterol and a PEGylated lipid (Rauch et al, 2020). The CVnCoV can be stored in normal refrigerator temperatures of +5°C for three months and in room temperature for 24 hours.[39]

The CVnCoV vaccine has been shown to elicit neutralizing antibodies against SARS-CoV-2 as well as T-Cell responses in rodents (Rauch et al, 2020). In human subjects the CVnCoV vaccine elicits IgG responses against the RBD and spike protein as well as neutralizing antibodies against SARS-CoV-2 (Kremsner et al, 2020).

[39] CureVac. COVID-19 [Internet]. Last updated: February 12, 2021 [cited 2021 Mar 20]. Available from URL: https://www.curevac.com/en/covid-19/

3.7.3. Gamaleya: Sputnik V

The Russian Sputnik V vaccine is a non-replicating viral vector vaccine that is developed from a recombinant adenovirus serotype 26 (rAd26) vector and recombinant adenovirus serotype 5 (rAd5) vector that contain the genetic material of the target SARS-CoV-2 spike protein (Poland et al. 2020). The use of two different adenovirus vectors to carry the gene for coding the spike protein, differentiates Sputnik V vaccine compared to other adenovirus vaccines.[40] A single 0.5 ml dose contains 10^{11} viral particles which are administered intramuscularly (Logunov et al, 2021). The injections are given in two doses separated by 21 days each containing a different adenoviral strain (Logunov et al, 2021). The first injection contains the rAd26 strain and the second dose contains the rAd5 strain (Logunov et al, 2020). The Sputnik V vaccine can be stored at –18°C.[41]

The Sputnik V vaccine regimen has been shown to elicit immunological responses in human subjects by producing neutralizing antibodies, IgGs and T-Cell responses against SARS-CoV-2 (Logunov et al, 2020). In a placebo-controlled phase 3 clinical trial the Sputnik V vaccine showed 91.6% efficacy against COVID-19 (Logunov et al, 2021).

[40] Gamaleya National Center of Epidemiology and Microbiology. About Vaccine [Internet]. [cited 2021 Mar 14]. Available from URL: https://sputnikvaccine.com/about-vaccine/

[41] Jones I, Roy P. Sputnik V COVID-19 vaccine candidate appears safe and effective [Internet]. The Lancet; 2021 [cited 2021 Feb 17]. Available from URL: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)00191-4/fulltext

CHAPTER 4

DISCUSSION

4.1. Risks of vaccines and pharmacovigilance

Vaccines, similarly to all medicines are known to cause adverse reactions. Vaccines rarely cause serious side-effects but commonly reported mild symptoms are pain, swelling, redness, fever, chills, lethargy, headache and muscle and joint pains.[42] The COVID-19 vaccines examined in this study appear to cause similar mild and transient adverse reactions like other vaccines. There are still risks for more serious adverse reactions such as anaphylaxis resulting from vaccination that can be lethal (McNeil et al, 2016). Anaphylaxis from vaccination is not necessarily caused by the active ingredient in the vaccine but can be caused by residues or other components in the product. With COVID-19 vaccinations, preparations should be taken in case of anaphylactic reactions.[43] These preparations include having epinephrine and antihistamines available at the vaccination site as well as having the personnel trained to recognize early signs of anaphylaxis.[43] The vaccination process in itself is often a source of adverse effects in the form of anxiety and possibly fainting (Siegrist, 2007). These reactions are of psychological origin and not a consequence of the medicinal product. Measures should be still taken to avoid such reactions as they might lead to further injuries due to falling.

Some adverse reactions do not appear immediately after the vaccination and are therefore harder to associate with the vaccine. One recent example of such delayed and unexpected serious adverse reaction was narcolepsy caused by the Pandemrix vaccine against the H1N1-virus also known as swine flu in 2009 (Sarkanen et al, 2018; Hallberg et al, 2019). The Pandemrix vaccine was given to millions of people in Europe which led to increases in new

[42] U.S. Department of Health & Human Services (HHS). Vaccine Side Effects [Internet]. [cited 2021 Jan 6]. Available from URL: https://www.vaccines.gov/basics/safety/side_effects

[43] Centers for Disease Control and Prevention (CDC). Management of Anaphylaxis at COVID-19 Vaccination Sites [Internet]. Last updated: February 10, 2021 [cited 2021 Mar 23]. Available from URL: https://www.cdc.gov/vaccines/covid-19/clinical-considerations/managing-anaphylaxis.html

cases of narcolepsy emerging from Finland and other countries in Europe, particularly in children (Sarkanen et al, 2018). Cases such as this highlight the need for careful monitoring of adverse reactions after the clinical trials and marketing authorization of new vaccines.

Monitoring of adverse reactions is part of pharmacovigilance which is the science and activities aiming to prevent drug related adverse effects and monitoring the efficacy safety balance of a drug before and after it enters the market.[44] The European Medicines Agency (EMA) started a pharmacovigilance plan specifically for the new COVID-19 vaccines applying the knowledge added from the 2009 H1N1 pandemic (EMA, 2020). This plan includes additional monitoring of spontaneous reporting of adverse reactions and monthly summary safety reports in addition to the usual Periodic Safety Update Reports (PSUR). It is crucial that healthcare providers and the general public monitors possible adverse reactions and that any adverse reactions are reported properly via Individual Case Safety Reports (ICSR) (EMA, 2020). Even if adverse reactions are reported after COVID-19 vaccinations, it does not always mean that the vaccine has caused the adverse reaction. The reported adverse reaction has to be assessed by trained professional to evaluate the relationship between the adverse reaction and the vaccine by using a causality assessment algorithm (EMA, 2017). One such algorithm is the French Method, which assesses the chronological and semiological likelihood of the vaccine to be the cause of the adverse reaction (Mann and Andrews, 2007).

[44] European Medicines Agency (EMA). Pharmacovigilance: Overview. [Internet]. [cited 2020 Nov 5]. Available from URL: https://www.ema.europa.eu/en/human-regulatory/overview/pharmacovigilance-overview

4.2. Myths about vaccines and the antivaccination movement

It is clear that no matter how effective and safe the COVID-19 vaccines are, they will not work unless people take them. Although, there are risks with the COVID-19 vaccines like with any other vaccine, the general public might not be able to assess the risk benefit relationship of the vaccination. The situation is further exacerbated by an active antivaccination movement campaigning against COVID-19 vaccines which can potentially further distort the perspectives of the general public (Burki, 2020). The concerns regarding vaccines commonly include that they cause various diseases such as autism, attention deficit disorders, autoimmune disorders and sudden infant death syndrome (Wolfe et al, 2002) Although, concerns about vaccines are amplified in modern times due to the internet and social media, the phenomenon in itself is nothing new (Wolfe et al, 2002, Petousis-Harris and Alley, 2020). Already during the early smallpox vaccination programs, there was resistance towards compulsory vaccines.[45] Some examples of the real harm that these antivaccination campaigns can cause are the low HPV vaccine coverage in Japan and a measles outbake in Samoa which led to the death of 83 people (Petousis-Harris and Alley, 2020). The WHO declared vaccine hesitancy as one of the ten threats to global health in 2019.[46]

[45] The College of Physicians of Philadelphia. History of Anti-vaccination Movements [Internet]. Last updated: January 10, 2018 [cited 2021 Mar 23]. Available from URL: https://www.historyofvaccines.org/content/articles/history-anti-vaccination-movements

[46] World Health Organization (WHO). Ten health issues WHO will tackle this year [Internet]. [cited 2021 Mar 23]. Available from URL: https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

4.3. Other reviews about the safety and efficacy of COVID-19 vaccines

An early review about SARS-CoV-2 and clinical trials on the potential treatment strategies, concluded that vaccines should be considered as a long-term solution for tackling the pandemic (Tu et al, 2020). In addition to vaccine candidates, they reviewed other treatment candidates including favipiravir, hydroxychloroquine and remdesivir which the authors considered to be more viable as a rapid response to COVID-19. In June 2020 the EMA granted a conditional marketing authorization to remdesivir for COVID-19 treatment in pneumonia patients from 12 years of age pneumonia who need supplemental oxygen.[47] Another review on the COVID-19 vaccines candidates was conducted by searching ClinicalTrials.gov database where the authors found more than 200 registered clinical and preclinical trials (Biała et al, 2021). Their paper included many of the same vaccines that was included in our review such as the BNT162b2, ChAdOx1 nCoV-19, The Sputnik V and mRNA-1273. A systematic review was conducted that examined results from clinical trials about the COVID-19 vaccine candidates (Dong et al, 2020). They included trials examining the mRNA vaccines, inactivated vaccines and Non-replicating vector vaccines. Their conclusions were comparable to this study in that most vaccines elicited immunologic responses such as neutralizing antibodies against SARS-CoV-2 in the study participants (Dong et al, 2020).

Given that advanced age is a risk factor for COVID-19, it is important to consider the safety and efficacy of COVID-19 vaccines in this population (Jordan et al, 2020). A review assessing COVID-19 vaccines and their evidence in older adults highlighted that the vaccines tend to cause less adverse reactions in older adults while still being effective (Teo, 2021). In

[47] European Medicines Agency (EMA). First COVID-19 treatment recommended for EU authorisation [Internet]. EMA; 2020 [cited 2021 Mar 24]. Available from URL: https://www.ema.europa.eu/en/news/first-covid-19-treatment-recommended-eu-authorisation

a commentary article about the efficacy and safety of COVID-19 vaccines in older people the authors emphasized that although, older people have been included in the clinical trials, there is still a lack of data in older patients with comorbidities and frailty (Soiza et al, 2021).

4.4. Limitations

This study has multiple limitations. The literature search on the safety and efficacy of COVID-19 vaccines only included 3 different databases, which excludes any publications not included in these databases that otherwise would have met the inclusion criteria. Also, only publications in the English language were included in this study. Only placebo-controlled human studies were included in the review. Any relevant data from open label studies with humans or animals about the safety and efficacy of COVID-19 vaccines are not included in the review. Another limitation in this study was that no systematic method of harmonizing the information about the clinical trials was used. The data is presented in a descriptive manner and no statistical analysis was conducted. The total number of studies reviewed was not high (N=14).

There is also a total lack of safety and efficacy data in this study about COVID-19 vaccines in pregnant and breastfeeding women. There is an urgent need to conduct studies in this population to assess how are the mother and the developing infant affected by the vaccines. It should be also assessed to what degree the vaccine components are excreted to the milk of a breast-feeding women and how they it might affect the health of the infant. In 12 March 2021 BioNTech SE is recruiting participants to assess the safety and immunogenicity of their BNT162b2 vaccine in healthy pregnant women.[48] Janssen has also registered an clinical trial

[48] BioNTech SE. Study to Evaluate the Safety, Tolerability, and Immunogenicity of SARS CoV-2 RNA Vaccine Candidate (BNT162b2) Against COVID-19 in Healthy Pregnant Women 18 Years of Age and Older [Internet]. [cited 2021 Mar 24]. Available from URL: https://clinicaltrials.gov/ct2/show/NCT04754594?term=pregnant&type=Intr&cond=covid-19&draw=2&rank=2

for their Ad26.COV2.S vaccine in pregnant women.[49] None of the studies in this review included people under the age of 16 and therefore there is need for data for this demographic as well.

The second part of the study reviewed the characteristics of 7 different COVID-19 vaccines that are either authorized for use in the European Union or are under rolling review by the EMA. There are many more COVID-19 vaccines that are authorized for use in other parts of the world which are not reviewed in this study.[13] Therefore, this review does not give a complete picture of all the available COVID-19 vaccines. Similarly, to the clinical trials on safety and efficacy of COVID-19 vaccines, no systematic method of analyzing the data was used.

4.5. Conclusion

The results from this study suggest that COVID-19 vaccines elicit mainly mild and transient adverse effects similarly to other vaccines. All the vaccines included in the literature review appear to produce either immunologic responses to the vaccine or efficacy against COVID-19. More data is needed about the safety and efficacy of COVID-19 vaccines in special populations such as pregnant women and children. There are currently 4 vaccines authorized for use in the European Union via conditional marketing authorization. These vaccines are: Comirnaty, mRNA-1273, AZD1222 and Ad26.COV2.S. Three more vaccines are under rolling review of the EMA. These vaccines are the NVX-CoV2373, CVnCoV and Sputnik V.

[49] Janssen. A Study of Ad26.COV2.S in Healthy Pregnant Participants (COVID-19) (HORIZON 1) [Internet]. [cited 2021 Mar 24]. Available from URL: https://clinicaltrials.gov/ct2/show/NCT04765384?term=pregnant&type=Intr&cond=covid-19&draw=2

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Appendix 1:

Ethics Approval

L-Università ta' Malta

Ville Juhani Vainionpaa <ville.vainionpaa.19@um.edu.mt>

FRECMDS_2021_063 - 7699_30012021_Ville Vainionpää - for records

FACULTY RESEARCH ETHICS COMMITTEE <research-ethics.ms@um.edu.mt> 3 February 2021 at 10:10
To: Ville Juhani Vainionpaa <ville.vainionpaa.19@um.edu.mt>
Cc: Serracino Inglott Anthony at Medicines Authority <anthony.serracino-inglott@gov.mt>, Nicolette Sammut Bartolo <nicolette.sammut-bartolo@um.edu.mt>

Dear Ville Vainionpää,

Since your self-assessment resulted in no issues being identified, FREC will file your application for record and audit purposes but will not review it.

You may proceed with your study.

Any ethical and legal issues including data protection issues are your responsibility and that of your supervisor.

Kindly **confirm** that you sent all the documents which you attached to the UREC form and also other documents related to your study.

These documents are also requested for audit purposes.

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Appendix 2:

Main adverse effects resulting from the studies

Number	Main adverse reactions	Serious adverse reactions	Reference
1.	Pain, redness, swelling, itching, coughing, diarrhea, fatigue, fever, headache, nausea and vomiting, pruritus	No serious adverse reactions reported	Xia et al, 2020
2.	Pain, induration, redness, swelling, itch, fever, headache, fatigue, vomiting, diarrhea, muscle pain, joint pain, oropharyngeal pain, cough, nausea, dyspnea, appetite impaired, mucosal abnormality, pruritus	No serious adverse reactions reported	Zhu et al, 2020
3.	Pain, tenderness, warmth, redness, swelling, itch, chills, fatigue, fever, feverish, joint pain, malaise, muscle ache, nausea	No vaccine related serious adverse reactions reported	Folegatti et al, 2020
4.	Pain, redness, swelling, fever, fatigue, headache, chills, diarrhea, muscle pain, joint pain, medication use	No serious adverse reactions reported	Mulligan et al, 2020
5.	Erythema or redness, induration or swelling, pain, tenderness, arthralgia, fatigue, fever, headache, myalgia, nausea, malaise	No serious adverse reactions reported	Keech et al, 2020
6.	Pain at the injection site, redness, swelling, fever, fatigue and chills	No serious adverse reactions reported	Walsh, et al 2020
7.	Pain, tenderness, warmth, redness, swelling, itch, induration, chills, fatigue, fever, feverish, joint pain, malaise, muscle ache, nausea	No vaccine related serious adverse reactions reported	Ramasamy et al, 2020
8.	Pain at the Injection site, redness, swelling, fever, fatigue, headache, chills, vomiting, diarrhea, muscle pain, joint pain and the use of antipyretic medication	Shoulder injury from injection, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia and right leg paresthesia.	Polack et al, 2020
9.	Pain, swelling, itch, redness, fever, fatigue, inappetence, nausea, constipation, mucocutaneous abnormalities, headache, vomiting	No serious adverse reactions reported	Xia et al, 2021
10.	COV003 and COV005 not available from the publication.	Two possibly vaccine related serious adverse reaction were reported:	Voysey et al, 2021

		transverse myelitis and ever higher than 40°C	
11.	Pain, swelling, redness, discoloration, pruitus, fatigue, diarrhea, fever, headache, cough, hypersensitivity, decreased appetite	No vaccine-related serious adverse events were reported	Zhang et al, 2021
12.	Pain, erythema, swelling, lymphadenopathy, fever, headache, fatigue, myalgia, arthralgia, nausea or vomiting, chills	Serious adverse reactions were rare and had similar frequencies between vaccine and placebo groups	Baden et al, 2021
13.	Pain, redness, swelling, fever, headache, myalgia, diarrhea, nausea, vomiting, fatigue	No vaccine related serious adverse reactions reported	Richmond et al, 2021
14.	Flu-like illness, injection site reactions, headache and asthenia	No vaccine related serious adverse reactions reported	Logunov et al, 2021