To be or not to be: The heterologous prime-boost vaccination against SARS-CoV-2

University of Groningen

Sanne Wind S3664481

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Biomedical sciences RUG

Supervised by Prof. dr. W. Helfrich

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Content

Abstract

Due to the pandemic caused by the novel coronavirus SARS-CoV-2, the world is in great need for protective therapeutics resulting in at least four vaccines that are currently clinically applied in European and American vaccination programs. However, due to the unequal distribution and inconclusiveness concerning the efficiency of the vaccines, alternative vaccination schedules could be of great importance. Previously, heterologous prime-boost regimes have demonstrated beneficial effect in the vaccination against other challenging infectious diseases and thus may be applicable to enhance or facilitate vaccination against SARS-CoV-2.

This paper will discuss the current knowledge of SARS-CoV-2, the immune response upon infection, the concept of heterologous prime-boost approaches, and how the latter may be applied to clinical use. Preliminary data from phase 2 clinical trials on heterologous prime-boost schedules demonstrated the induction of high neutralizing antibody titers and a prominent T cell response, indicative of its protective activity. A randomized controlled trial compared the immunogenicity of a heterologous regime to a homologous regime. The results demonstrated a robust immune response in both the homologous regimes. Especially the T cell response of the Oxford/AstraZeneca-Pfizer/BioNTech regime, which increased by 2-fold, shows potential for a more broad immune response compared to the homologous regimes.

Importantly, there appear to be no safety concerns when administrating heterologous vaccines. Therefore, vaccination rollout can become more flexible. After the results of the randomized trial were published, various government institutions across Europe gave advice to incorporate the heterologous regime into vaccination programs, whereby individuals are primed with Oxford/AstraZeneca and then boosted with Pfizer/BioNTech.

Introduction

On December 31, 2019, several cases of pneumonia of unknown cause were reported by Wuhan Municipal Health Commission. Patients clinically presented with symptoms similar to pneumonia caused by viral infections (1). As of the 9th of January 2020, the Chinese authorities determined the causative agent of the outbreak, a novel coronavirus tentatively named 2019-nCov (2). Research based on characteristic features, such as phylogeny and taxonomy, revealed that 2019-nCov forms a sister clade to the Severe acute respiratory syndrome coronaviruses (SARS-CoVs) and was therefore later denoted as Sars-Cov-2 (3). Infections caused by coronaviruses often result in mild disease only. However, as demonstrated by two other human coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV)(4,5) and Middle east respiratory syndrome coronavirus (MERS-CoV)(6), the arrival of novel coronaviruses can give rise to new alarming epidemics. Combined, SARS-CoV and MERS-CoV accounted for over 10.000 reported cases in humans. COVID-19 is one of the latest addition to the list of alarming epidemics and is subsequently declared as a pandemic by the World Health Organization (WHO) on march 11, 2020 (7).

Whereas the other two human coronaviruses kept the number of cases somewhere in the thousands, SARS-CoV-2 already accounts for over 170 million reported cases of which 3.7 million resulted in death (8). Although the mortality rate of COVID-19, which is estimated to be somewhere around 3% (9), is considerably lower than observed in SARS and MERS, with a mortality rate of 9.6% and 35.5% respectively(10)(11), the novel coronavirus appears to be much more virulent compared to the former viruses (12). In addition, the number of infection individuals continues to grow with each day, indicating the predominate position of the virus in relation to humans. With this exceptional high number of cases and the numbers still rising, the highest priority is to obtain herd immunity against the virus, thereby reducing the opportunity of spreading the virus and therefore the cases of infection.

Herd immunity is a form of indirect protection from infectious diseases and can be easily achieved if a sufficient percentage of the population has obtained immunity to the causative pathogen. Immune individuals are not likely to be involved in transmission of the disease, therefore interfering with further infection, resulting in a decrease in spread or even eradication of the disease (13). However, the acquired immunity to SARS-CoV-2 after infection is still a matter of uncertainty and controversy, especially the duration of the humoral immunity against the virus. The IgG anti-SARS-CoV-2 antibody titres seem to experience a rapid reduction in the first months after infection (14). One study reports that the 5 months following SARS-CoV-2 infection associated with an 83% decrease in risk of infection, indicating the possibility of reinfection (15). Another study demonstrated a more gradual decline in antibodies, with antibodies detectable for at least 11 months after infection (16). Furthermore, if infection does indeed results in long-lived immunity, acquiring herd immunity in the natural way, meaning a large fraction of the population must undergo infection first, would cause irresponsible deathrates, long-term disabilities and pressure on healthcare systems (17,18).

For these reasons, acquiring herd immunity using vaccination programs provides the best outcome. The genetic sequence of SARS-CoV-2, published by the WHO on 11th of January 2020, gave rise to global activities concerning the development of vaccines.

The rapid response of China to the outbreak resulted in numerous studies which provided information about the structure of the novel virus (19). One of the studies led to the identification of the spike protein and its essential role in the pathogenesis of the virus (20). The spike protein is crucial for cell entry of the SARS-CoV2 virus and in initiating an immune response. Other viral structures are the envelope, membrane and nucleocapsid proteins (21).

As of June 2021, at least 287 vaccines are being evaluated in trials, either at a pre-clinical or clinical stage, varying from mRNA vaccines to whole inactivated SARS-CoV-2 and more (22). However, only a small fraction of all the candidate vaccines are currently listed by the WHO for emergency use, of which four have been approved by the European Medicines Agency (EMA) (23). Pfizer/BioNTech, Moderna, Oxford/AstraZeneca and Janssen are the four vaccines currently used in the European vaccination program. All four vaccines initiate the production of the spike protein, thereby triggering a neutralizing immune response against the viral structure (24).

However, the vaccines differ in mode-of-action. Whereas Pfizer/BioNTech and Moderna are mRNA vaccines, Oxford/AstraZeneca and Janssen are vector vaccines. Despite the fact that both delivery systems generate significant neutralizing antibody titres, the vector vaccines show less protection. The vector vaccines demonstrated an efficiency of approximately 70%, whereas protection efficiency by the mRNA vaccines is as high as 90-95% (25-28). Of note, a single dose of the Janssen vaccine is sufficient to induce immune response robust enough for protective immunity, whereas the other vaccines induce a relative weak immune response when administered a single dose and thus need a second dose to induce sufficient antibody titers (29).

Currently, most individuals are to be vaccinated with two doses of the same vaccine. A study in rhesus macaques demonstrated evidence of a great increase in immunogenicity by using a strategy called heterologous prime-boost (30). The strategy involves priming and boosting of immunity with vaccines that differ in mode of action but are directed at similar or identical antigens. As seen in vaccines against other infectious diseases, such as Ebola and HIV-1, this heterologous prime-boost strategy appears to exert similar positive effects as demonstrated in the study in rhesus macaques (31,32).

Except for the Janssen vaccine, the current vaccines used in the vaccination program require an additional booster to induce a sufficient neutralizing antibody titers. As the heterologous prime-boost strategy demonstrated beneficial effects on immunogenicity in animal studies and other infectious diseases, it can be argued to exert similar effects on the immunogenicity against SARS-CoV-2. Therefore, in this study the effect of the heterologous prime-boost vaccination against SARS-CoV-2 will be analyzed and discussed.

Background of SARS-CoV-2

The first reported cases of pneumonia of unknown etiology, identified in local hospitals, were all linked to the Huanan Seafood Wholesale Market. Although the origin of the novel coronavirus is still a matter of uncertainty, the evidence linking the virus to the wet food market in Wuhan starts to accumulate (33). Coronaviruses are naturally present in bats (34), and previous research has demonstrated the potential of various bat SARS-CoVs to be transmissible to humans (35). The novel coronavirus is likely due to a spillover of a zoonotic disease, involving bats as natural reservoir (36). The former human coronaviruses, SARS-CoV and MERS-CoV, presumably originated from bats as well. Furthermore, various related coronaviruses are discovered in diverse bats around the world, supporting the concept of bats as the natural reservoir for potential human coronaviruses (37).

SARS-CoV-2 and SARS-CoV share approximately 80% of their genome sequence and share similarities concerning the initiation of their virulence, as both initial cases emerged during the winter period and are associated with contact to live animals at Chinese animal markets (38). The MERS-CoV is expected to differ in viral evolution to SARS-CoV-2, but still shares approximately 50% sequence identity with SARS-CoV-2 (39). SARS-CoV-2 belongs to the family of *Coronaviridae* and the order Nidovirales. Four different genera exist in the family, the alpha-, beta-, gamma-, and delta-CoVs. The pathogenic viruses, with potential to infect humans, including SARS-CoV, MERS-CoV and SARS-CoV-2, belong to the genus *Betacoronavirus* (40,41).

The coronaviruses are non-segmented positive-sense RNA viruses and present with a lipid bilayer envelop (42). The viruses express a characteristic set of structural proteins, embedded within the lipid envelop, consisting of the envelop (E), membrane (M) and spike (S) structural proteins, expressed with a 1:20:300 ratio, respectively(55). The fourth structural protein is the nucleocapsid protein (N), which encapsulates the viral genome. The name corona originates from the Latin translation for crown, referring to the spike protein (S protein), which presents in a crown like structure around the virus particles. A single spike glycoprotein, present on the viral envelop, consists of three monomers, each monomer consisting of a S1 subunit and a S2 subunit.

The homo-trimeric protein is essential for cell entry and is therefore arguably the most important feature in the pathogenesis and virulence of the virus (43). The surface unit, the S1 subunit of a S protein monomer, is responsible for binding to cellular receptors, thereby facilitating attachment to the target cells (4). Furthermore, upon cell entry, the S protein must be primed by cellular proteases, implying cleavage of the S protein at the S1/S2 and S2'site, thereby allowing fusion of both cellular and viral membranes. The S2 subunit exerts its function at the last step of cellular entry. The pathogenesis of SARS-CoV-2 in humans mandate the angiotensin converting enzyme 2, ACE2 (44), as cellular receptor to enable cell entry and TMPRSS2, a cellular serine protease which the virus utilizes for cleavage and therefore priming of the S protein (45). ACE2 is a surface enzyme which negatively regulates the renin-angiotensin system (RAAS), thereby regulating vasodilation. A study in response to first epidemic, due to SARS-CoV, demonstrated the expression of surface ACE2 protein on lung alveolar epithelial,

thereby indicating the entry route for SARS-CoV (46). Further research supported this notion and indicated the expression of the viral entry-associated protease TMPRSS2 in nasal goblet and ciliated cells as well (47). Worth mentioning is the increased affinity of SARS-CoV-2 for ACE2 than its predecessor SARS-CoV, which possibly facilitates in the human transmission events, resulting in the exceptional high number of cases compared to SARS outbreak in 2002 (48).

After cellular entry, the viral RNA genome is released into the cytosol, whereafter translation is initiated. The genome contains seven conserved open reading frames (OFRs) among coronaviruses, in the following order: 5'-ORF1a-ORF1b-S-ORF3-E-M-N-3'. Four of the ORFs encode for the for the structural proteins E, M, N and S (49). The first ORF, which covers approximately two-third of the genome, is a 5' frameshifted polyprotein. Initiation of translation starts at the first ORF, ORF1a. After a frameshift signal, translation continues in ORF1b, resulting in a continues production of ORF1a and ORF1ab polypeptides, or pp1a and pp1ab. Pp1a and pp1ab are viral replicase proteins, which in turn form an active protein complex involved in the transcription and replication of viral RNAs.

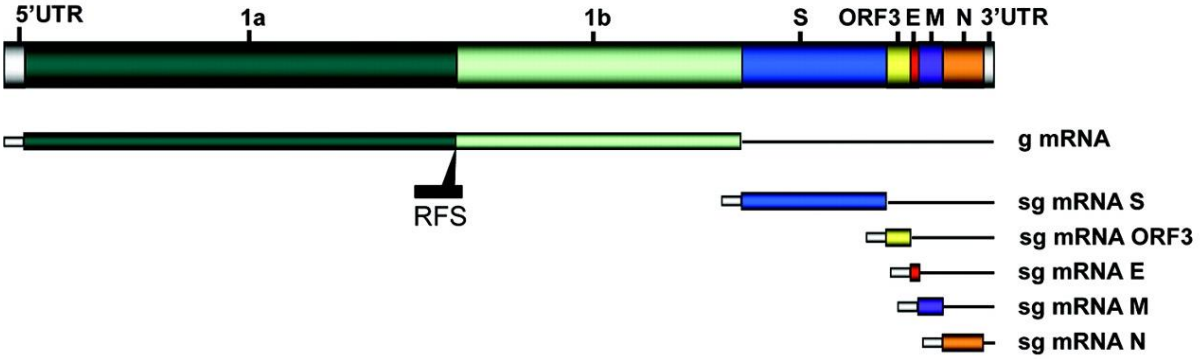

***Figure 1**.The seven conserved open reading frames among coronaviruses. (from Pyrc et al, 2020)*

After translation, the polypeptides are cleaved into 16 individual non structural proteins by virus-encoded proteases, which eventually form the RNA-dependent RNA polymerase allowing for the viral genome to replicate (50). Coronaviruses are reliant on the RNA-dependent RNA synthesis for replication of the viral genome for obtaining multiple copies of the genomic RNA and for transcription of the sub genomic mRNA (sgmRNA), which encodes for the viral structural proteins (51). The structural and accessory proteins, translated from the sgmRNA, are loaded into the ER-Golgi intermediate compartment, whereafter the virions are assembled. Lastly, the transcribed RNA genomes are transferred into the novel virions, after which the virions are released into the extracellular environment, thereby initiating a new lifecycle of SARS-CoV-2 (52).

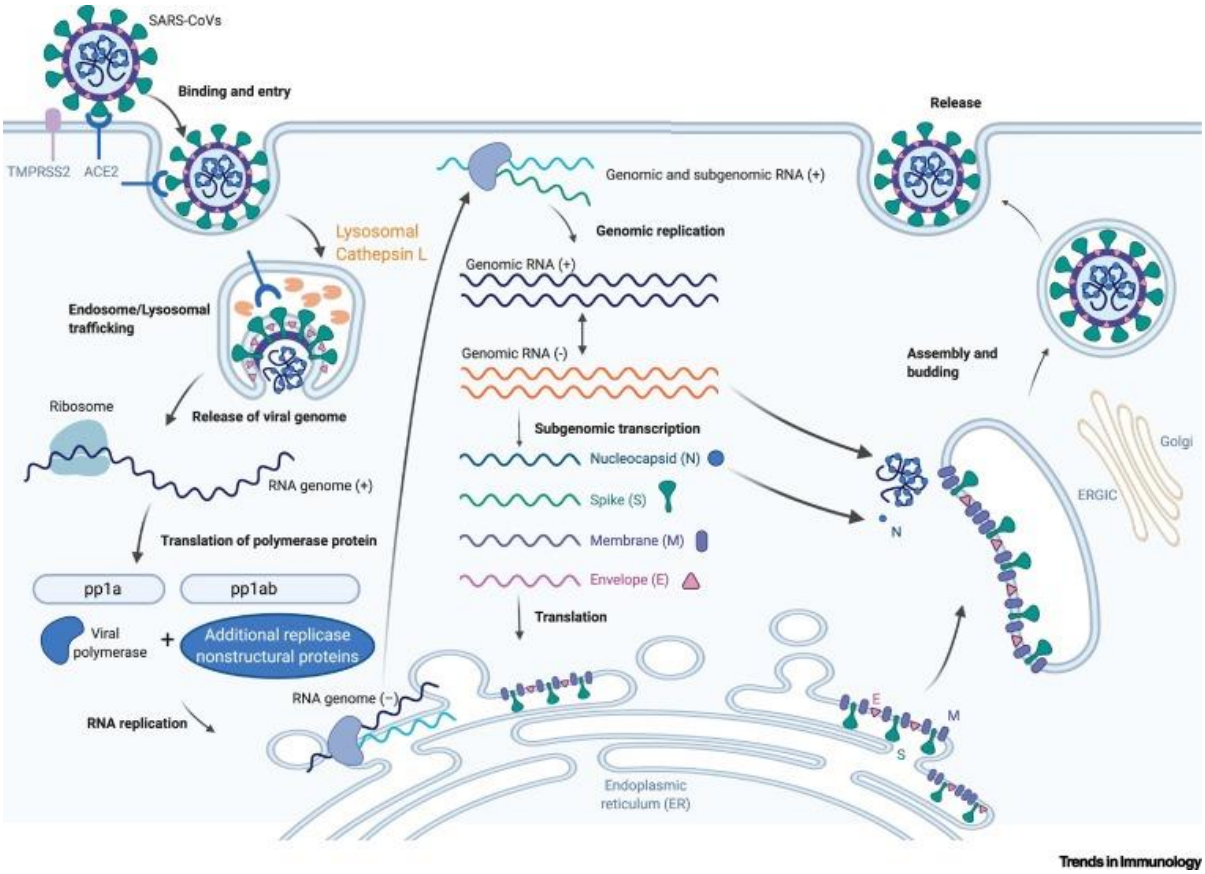

***Figure 2**.The Severe Acute Respiratory Syndrome Coronavirus 2 Lifecycle. (from Harrison et al, 2020)*

Immune response upon infection

Upon entry, in the epithelial cells of the nasal cavity, replication of the viral genome is initiated. The first 1-2 days are asymptomatic, due to the relatively low hindrance caused by the innate immune cells (53). In this period, the virus replicates and therefore multiplies, whereafter the virus migrates to the lower respiratory tract, triggering a strong immune response. The immune response is initiated by the innate immune system, whereby the antigen presenting cells recognize the PAMPs, in the case of SARS-CoV2 comprising nucleic acids, glycoproteins, lipoproteins and additional small molecules present in the virus. The pathogenesis of SARS-CoV-2, in reaction to the rapid replication, transcription and assembly, is similar to the former SARS-CoV, since both are predominantly mediated by cytokine release (54). The replication mechanism of the virus inside the cellular environment of the host results in epithelial and endothelial cell apoptosis and increased vascular permeability. Due to this, pro-inflammatory cytokines and chemokines are released, resulting in an acute inflammatory and immune response (55).

In individuals infected with a previous human coronaviruses, SARS-CoV, the dysregulation of the immune response appeared to be the factor resulting into disease, rather than the level of viremia. The infected individuals expressed insufficient amount of type I interferon (IFN), unbalanced quantities of proinflammatory cytokines and displayed dysfunction of the T lymphocytes, CD4+ and CD8+ (56). Particularly, the impaired functioning of the innate immune cells of the host affect the course of disease,

due to the overall effect on the cytokine production (53). The same elevated levels of cytokines are observed in individuals infected with SARS-CoV-2, including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A and TNF-α (57). The proinflammatory cytokine storm is critical for the pathogenesis of SARS-CoV and even associates with the severity and related mortality of the disease (58). As seen in SARS and MERS, the alveolar damage is a result of the exceptional high levels of proinflammatory cytokines. The same effect is observed in patients infected with SARS-CoV-2 (59). A schematic overview of the dysregulated immune response displayed in figure 3.

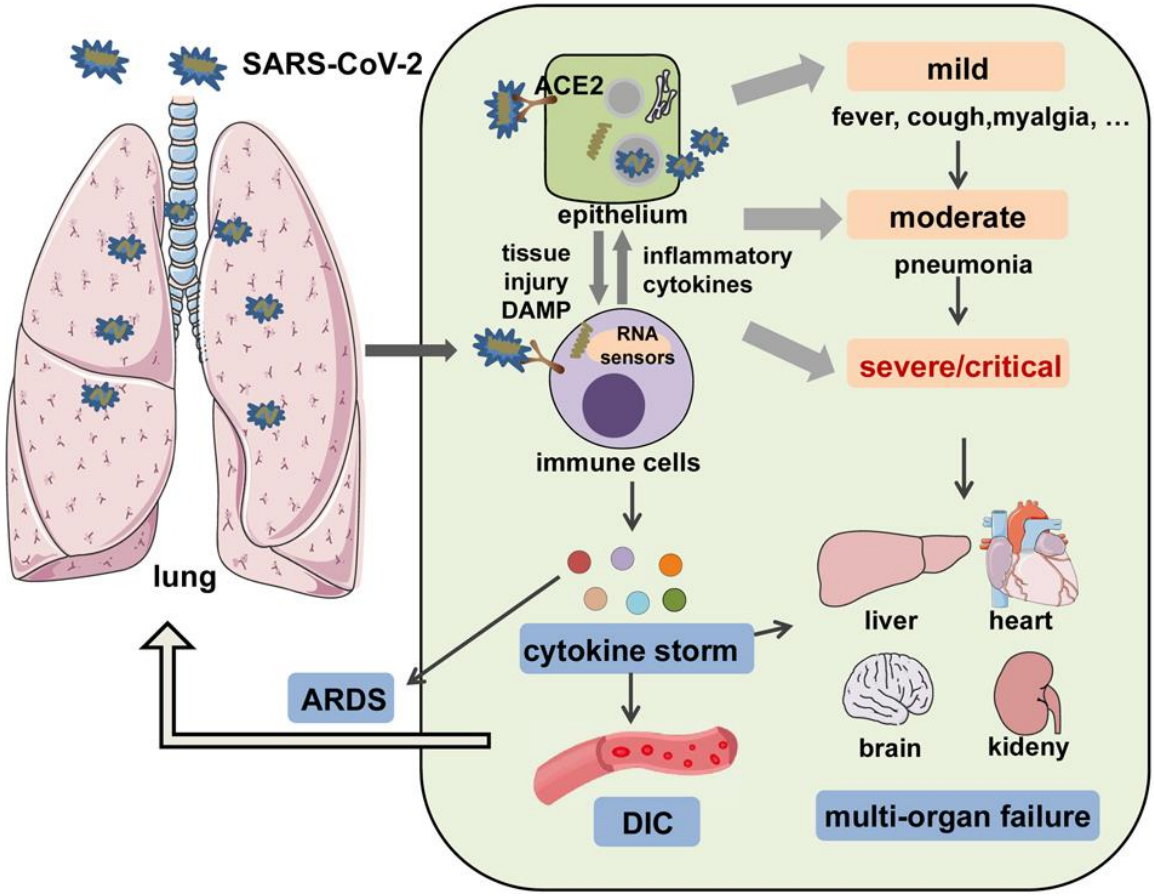

***Figure 3**.SARS-CoV-2 enters human airway epithelial or immune cells by binding to ACE2 receptors, resulting in tissue damage, DAMPs and cytokine production. (From Yang et al, 2021)*

The adaptive immune response displayed improper functioning as well during the course of infection, however the severity of disease appears to be correlated to the dysfunction. In asymptomatic and non-severe disease of patients infected with SARS-CoV-2, the adaptive immune response appears to function properly. By recording the immune cell population, the presence of antibody-secreting cells, follicular helper T cells, activated CD4+ and CD8+ T cells was observed. Furthermore, IgM and IgG were detected, indicating the presence of humoral memory against the virus (60). However, in patients with severe COVID-19, the T cell counts are significantly reduced.

Additionally, the surviving T cells appear to be functionally exhausted, indicating the dysfunction of the adaptive immune response in severe disease (61). For the production of serum antibodies, B cells are required. B cells must be activated before

proper antibodies can be produced. Naïve B cells interact with activated T cells, resulting in activation of the B cell and the production of antibodies (62). As the T cells display improper functioning in severe disease, it can be argued that the process of activating the B cells and therefore the production of serum antibodies does not result in proper immunity against SARS-CoV-2. Moreover, the life of the produced IgG and IgM is still indistinct, since the IgG anti-SARS-CoV-2 antibodies rapidly decline in the first months after infection and studies report controverse results (14-16).

Current vaccines against SARS-CoV-2

In reaction to the emerging pandemic, numerous scientific institutions started the rapid development of therapeutical agents to introduce immunity, resulting in 287 candidate vaccines currently on trial, as on June 2021 (22). At least four different vaccines are currently EMA approved and therefore used to vaccinate the European population. The four vaccines, Pfizer/BioNTech, Moderna, Oxford/AstraZeneca and Janssen, can be divided into two groups, based on their mechanism of action. The first two, Pfizer/BioNTech and Moderna, are mRNA vaccines, whereas the Oxford/AstraZeneca and Janssen vaccines make use of an innocent adenovirus vector.

The mRNA vaccines are a relatively new therapeutical approach as alternative for the conventional vaccines. The first study successfully demonstrated the use the novel technique in 1990 (63), however due to issues concerning the mRNA instability and delivery methods, did not further resulted in the development of novel mRNA therapeutics. Nevertheless, during the last decade, various new technologies were invented. Therefore, some of the former issues concerning mRNA as therapeutic agent could be resolved. The safety is one of the great advantages of the mRNA vaccines over other approaches. Compared to the live attenuated or DNA-based vaccines, there is no additional risk of infection or mutations caused by insertion (64). Another great advantage is the high efficiency of the mRNA vaccines, which translates into a 90-95% efficiency against SARS-CoV-2 (27,28). Both the Pfizer vaccine, BNT162b2, and the Moderna vaccine, mRNA-1273, are directed the same viral structure, the spike protein. Therefore, the mRNA molecule in both the vaccines encodes for the full-length spike of SARS-CoV-2.

Although both the vaccines make use of mRNA, the key differences lies in the delivery method. The mRNA of both vaccines is delivered in a nanoparticle, due to instability of the mRNA. It is possible to administer mRNA directly to individuals, however, no efficient immune response will be yielded. Uncovered mRNA molecules are rapidly degraded by extracellular RNases, ergo not able to reach the translation mechanisms and are therefore less sufficient (65). Furthermore, in order to reach the translation machinery, the mRNA must pass the lipid membrane first, a process in which the lipid nanoparticle facilitates (66). The composition of the nanoparticle differs between the Pfizer/BioNTech and the Moderna vaccine (Table 1). This difference in lipid delivery systems allows the Moderna vaccine to be stored and handled less harsh temperatures than the Pfizer/BioNTech vaccine (67).

Upon delivery of the mRNA into the cytosol, the encoded viral structure is translated, which in the case of COVID comprises the spike protein. The antigen integrates into

Name product	**Pfizer/BioNTech:** BNT162b2; Comirnaty	**Moderna:** mRNA-1273
Lipid nanoparticle components	0.43 mg ALC-0315 = (4-hydroxybutyl) azanediyl)bis (hexane-6,1-diyl)bis(2-hexyldecanoate) 0.05 mg ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N ditetradecylacetamide 0.09 mg 1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC) 0.2 mg Cholesterol	SM-102 (heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate} PEG2000-DMG = 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 1,2-Distearoyl-*sn*-glycero-3 phosphocholine (DSPC) Cholesterol

***Table 1**. Composition of nanoparticles COVID mRNA vaccines. (from Schoenmaker et al, 2021)*

the cellular membranes, inducing the adaptive immune response. Additionally, the RNA-expressed S is fragmented, whereafter the peptides can be presented at the cell surface. Therefore, a T-cell-mediated immune response is triggered as well. For this reason, vaccinated individual will initiate a broad immune response against the novel translated spike protein, resulting in immunity (68). Notably, both the vaccines induce a relative weak immune response when administered a single dose. Therefore, to obtain sufficient antibody titers, a second dose is needed (29).

Whereas the Pfizer/BioNTech and Moderna vaccines make use of mRNA to initiate an immune response, the Oxford/AstraZeneca and Janssen vaccines make use of viral vectors. Various vector-based vaccines against viral infections, such as HIV-1 and Ebola, have been examined in clinical trials (31,32). The concept of vector vaccines was firstly described in 1972, whereby recombinant DNA was created from the SV40 virus (69). A diverse series of viruses are currently employed for the use of constructing viral vector based vaccines, of which the Adenovirus (Ad) is the most common (70).

Both the vector-vaccines currently used in the vaccination program against SARS-CoV-2 employ Adenovirus vectors as well. The adeno vector vaccines are directed against the same antigen as the mRNA vaccines, the spike protein. The Oxford/AstraZeneca, AZD1222, consist of a replication-deficient chimpanzee adenoviral vector ChAdOx1, containing the gene encoding the SARS-CoV-2 spike protein (25). The Janssen vaccine, Ad26.COV2.S, consist of a replication-deficient human adenovirus type 26 vector Ad26, containing the gene encoding the SARS-CoV-2 spike protein as well. After delivery of the vector into the transfected nucleus, the viral structural protein of interest is translated. The transfected cells display the antigen to the antigen presenting cells, which in turn activate the adaptive immune system, resulting in the production of anti-SARS-CoV-2 antibodies, thereby introducing immunity to the virus.

Whereas the Oxford/AstraZeneca requires a booster dose, as required for both the mRNA vaccines as well, the Janssen vaccine is developed as a single shot vaccine. A

single-shot of the Janssen vaccine induces an immune response which is sufficient to obtain protective immunity, thereby lowering pressure on the vaccine supply (26). Currently, a second Phase 3 clinical trial is launched to investigate if the two-dose regime will result in better protection (71).

The use of vector vaccines has advantages over other vaccines. The adeno vectors are proven to induce a robust immune response, activating both the innate and adaptive immune system (72). Additionally, the stability of the adeno vector-vaccines is promising during pandemics. Whereas the adeno vector vaccines remain stable for 6 and 3 months at 2-8 degrees for Oxford/AstraZeneca and Janssen, respectively, the mRNA vaccines only remain stable for only a short period of 5 and 30 days at 2-8 degrees for Pfizer/BioNTech and Moderna, respectively (25-28). For this reason, the mRNA vaccines require ultra-cold chain storage, giving the adeno vector vaccines a major advantage over the mRNA vaccines concerning the transport during pandemics.

However, one of the disadvantages of employing the viral vectors of the Adenovirus is the possibility of pre-existing immunity. Since most individuals encountered a serotype of the adeno virus, administration of the viral vector can lead to severe outcomes. This was observed in an 18-year-old patient, who suffered from fatal systemic inflammatory response syndrome following adenoviral gene transfer (73). The issue concerning pre-existing antibodies is addressed by using a less prevalent adenovirus variants, such as the Ad26 vector used in the Janssen vaccine, or related adeno viruses from different species, such as the chimpanzee adenovirus vector deployed in the Oxford/AstraZeneca vaccine.

Additionally, the efficiency of the vector-based vaccines against SARS-CoV-2 is a limiting factor. Whereas the mRNA vaccines demonstrate an efficiency of 90-95%, the vector vaccines show a lower efficiency of approximately 70% (25-28). However notably, the adeno vector vaccines do result in similar efficiency of 85-90% at preventing severe illness as the mRNA vaccines do.

Heterologous prime-boost immunization

As stated in the sections above, the Pfizer/BioNTech, Moderna and Oxford/AstraZeneca vaccines require an additional booster dose, in order to produce sufficient antibody titers to obtain appropriate protective immunity against SARS-CoV-2. Currently, the prime-boost is homologous in that individuals receive a second dose of the same vaccine as their initial dose. Therefore, the pressure on the vaccine supply is increased, potentially leading to shortages of specific vaccines, which likely results in a delay of vaccine rollout. Due to the high medical and economical priority to achieve herd immunity, there is a growing demand for alternative strategies. Furthermore, the vector vaccines demonstrate a rather low efficiency compared to the opponent mRNA vaccines, which possibly can be improved by alternative vaccination approaches (25). A vaccination strategy employed in different infectious diseases is the heterologous prime-boost, whereby the subsequent boost immunization differs in delivery method from the initial prime immunization, however both directed at the same antigen.

The concept of heterologous prime-boosting was first reported in 1991, whereby mice primed with a live recombinant virus and boosted with a subunit recombinant protein were compared to either immunogen alone. The results indicated a more effective immunization when combining the immunogens compared to either immunogen alone (74). In 1992, a subsequent study successfully demonstrated the heterologous prime-boosting strategy in non-human primates, where protective immunity was achieved in rhesus macaques by priming the animals with a recombinant vaccinia virus expressing the gp160 antigen, whereafter the animals were boosted with a baculovirus producing the same gp160 (75). For various infectious diseases, for which previous vaccine development did not result in promising outcomes, the heterologous prime-boost strategy can be considered useful.

One of these challenging diseases is AIDS, caused by the human immunodeficiency virus type I (HIV-1). The idea of applying the prime-boost approach on the prevention of HIV-1 is first described in 1991 and based on the principle that traditional vaccines, such as subunit or inactivated, do not evoke an effective T cell response, whereas this T cell response is crucial for a proper immune response. As seen in HIV-1 vaccination, the recombinant envelope glycoprotein vaccine was able to produce specific neutralizing antibodies, however unable to evoke a cytotoxic T cell response. In contrary, the recombinant vaccinia expressing HIV-1 antigens was able to elicit sufficient T cell response, whereas it was unable to acquire sufficient antibody titers for protection (74).

Proceeding this rationale, numerous studies were conducted to examine the beneficial effects of prime-boost, thereby establishing the concept of the heterologous prime-boost approach as potential future for the HIV-1 prevention (76,77). In these studies, individuals were primed with ALVAC-HIV(vCP205), the recombinant canarypox vector vaccine containing genes encoding for HIV-1 antigens, and boosted with a vaccine containing either glycoprotein 120 or 160 subunit. Whereas in these studies, the approach did elicit a cellular and humoral immune response, the results were not optimal. Therefore, an additional trial examined the efficiency of the heterologous prime-boost approach on HIV-1 vaccination, in which the participants were primed with the ALVAC-HIV [vCP1521], the same recombinant canarypox vector vaccine as used in previous studies, and then boosted with AIDSVAX B/E, a glycoprotein 120 subunit vaccine (78). Although the study only demonstrated a mild benefit, the results were still promising.

Heterologous prime-boost against SARS-CoV-2

As demonstrated during the current pandemic, novel vaccines may not always be fully effective and therefore require finetuning, for which, due to an urgency, there is no time. The vector vaccine of Oxford/AstraZeneca demonstrated a 70% efficiency after the booster, which is still not optimal (25). Therefore, the heterologous prime-boost approach could potentially exert beneficial effects on the current pandemic. Ideally, by combining the vaccines that differ in mode of action, the protective efficiency of especially the adenovector vaccines would reach the same percentage as observed in the mRNA vaccinated individuals, or even higher.

An important factor to be considered when vaccinating in a heterologous prime-boost manner is the risk of vaccine-vaccine interactions, as this interaction can possible cause reduced immunogenicity. Vaccine-vaccine interactions can occur due to a chemical or physical interactions between the compounds of different vaccines, interactions between live vaccine or interference of the immune response (79).

A phase 2 trial investigated the effect on safety and efficiency of boosting individuals, who received a single dose of Oxford/AstraZeneca after 8 to 12 weeks, with a Pfizer/BioNTech mRNA vaccine (80). The results of the trial demonstrated an increase in IgG-RBD titres, the antibodies against the receptor-binding domain of the SARS-CoV-2 S protein. The IgG-RBD titres increased from the baseline of 71·46 binding antibody units per mililitre (the WHO international standard for anti-SARS-CoV-2 immunoglobulins, BAU/mL) to 7756·68 at the 14th day after administration with the Pfizer/BioNTech vaccine. The IgG titres against the trimeric S protein demonstrated an increase from 98·4 BAU/mL to 3684·87 BAU/mL. Additionally, in 100% of the participants neutralizing antibodies were present 14 days after administration of the Pfizer/BioNTech vaccine, in contrast to the initial 34.1% after a single dose of Oxford/AstraZeneca. Alongside the increase in humoral immune response, the cellular immunity increased by a 4-fold.

The study did not demonstrated serious adverse events, only mild and moderate reactions were observed. In conclusion, the study demonstrated that heterologous prime-boost approach in which individuals were primed with the Oxford/AstraZeneca vaccine and then boosted with the Pfizer/BioNTech results in a robust immune response with no or minor safety concerns. Notably, concrete data about the efficiency was not presented which would reveal any additional value of the heterologous prime-boost vaccinations on the protective efficiency.

An additional study, in which mice are vaccinated in a heterologous prime-boost regime, demonstrated promising results regarding the protective immunity (81). Of note, the four vaccines used in this study differ from the adenovector vaccines and mRNA vaccines currently used in the vaccination programs (Table 2). The results demonstrated an increase in neutralizing antibodies levels in animals primed with the adenovirus vectored vaccine and boosted with either the inactivated, recombinant RBD or mRNA vaccine.

Vaccines	Developer/Manufacturer	Platforms
BBIBP-CorV [8]	Beijing Institute of Biological Products/Sinopharm	Inactivated vaccine
Ad5-nCoV [14]	CanSino Biological nc./Beijing Institute of Biotechnology	Adenovirus type 5 vectored vaccine
ZF2001 [13]	Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences	Recombinant RBD vaccine
ARcoVax	People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech.	mRNA vaccine

Table 2. *The four vaccines used to vaccinate mice in a heterologous prime-boost regime, the developer and the mode of action (from Qian He et al, 2021)*

Additionally, the study observed a significant higher neutralizing antibody response in mice primed with the adenovirus vectored vaccine and boosted with an mRNA vaccine,

compared to the animals which received two doses of an mRNA vaccine only. This finding is of particular interest, since the current vaccines used in the vaccination programs rely on the same mode of action. Therefore, the increased neutralizing antibody response in mice after the heterologous vaccination of the adeno vector vaccines and mRNA vaccines may give an indication for the same outcome for humans. However, the results are limited as only the immune responses were measured, which do not necessarily translate to levels of protection. In addition to the increased neutralizing antibody response, the T cell response was further amplified in the group which received a heterologous prime-boost regime compared to those receiving a homologous prime-boost regime. Notably, the data described in mice do not necessarily corresponds to those for humans.

On the 25th of June 2021, results of a randomized controlled trial evaluating both reactogenicity and immunogenicity of a heterologous vaccination regime were published (82). In the trial, homologous and heterologous prime-boost regimes with an adenoviral vectored and mRNA vaccine against SARS-CoV-2 were compared.

Adults of the age 50 years or older, including individuals with comorbidities, were randomly assigned to eight different groups. Four groups were vaccinated in a homologous prime-boost schedule, receiving either the Oxford/AstraZeneca or Pfizer/BioNTech vaccine for both doses, administrated at intervals of 28- or 84-days. The other four groups received either a first dose of Oxford/AstraZeneca following a booster dose of Pfizer/BioNTech or a first dose of Pfizer/BioNTech following a booster dose of Oxford/AstraZeneca, both administrated at 28- or 84-day intervals. Notably, only the individuals with a 24-day prime-boost interval were reported in this study. The levels of SARS-CoV-2 anti-spike IgG and T-cell response were measured at 28 days after the booster dose.

The levels of anti-spike IgG observed in the Oxford/AstraZeneca – Pfizer/BioNTech group (12,906 ELU/mL) were higher compared to the homologous Oxford/AstraZeneca group (1,391 ELU/mL), meaning this heterologous prime-boost regime is non-inferior to a homologous regime using the adenovector vaccine. The Pfizer/BioNTech-AstraZeneca group demonstrated lower levels of anti-spike IgG (7,133 ELU/mL) than observed in the other heterologous schedule. Furthermore, the Pfizer/BioNTech-AstraZeneca heterologous schedule was not able to show non-inferiority against the homologous Pfizer/BioNTech group (14,080 ELU/mL). Strikingly, although the Pfizer/BioNTech-AstraZeneca schedule was not able to demonstrate non-inferiority, the anti-spike IgG levels in both heterologous regimes were higher than the homologous Oxford/AstraZeneca schedule.

Additionally, the T cell response observed in the heterologous Oxford/AstraZeneca-Pfizer/BioNTech group was 185 SFC/10^6 PBMCs (spot forming cells/10^6 peripheral blood mononuclear cells). This response is considerably higher compared to the other 3 regimes (50,80 and 99 SFC/10^6 PBMCs for the homologous Oxford/AstraZeneca group, the homologous Pfizer/BioNTech group and the heterologous Pfizer/BioNTech-Oxford/AstraZeneca group, respectively).

The study did report four serious adverse events, however none of which related to the vaccinations. In conclusion, this trial demonstrates that heterologous prime-boost regimes can be used with no or minor safety concerns, making the vaccination rollout of Oxford/AstraZeneca and Pfizer/BioNTech more flexible. Additionally, the data concerning the efficiency indicate that both heterologous prime-boost schedules result in an immune response that is more robust than the homologous Oxford/AstraZeneca regime.

Discussion

The novel coronavirus, SARS-CoV-2, easily expanded worldwide and accounts for over 170 million cases and 3.7 million deaths. These numbers continue to grow each day. Accumulating evidence about the origin of the novel coronavirus indicate the Huanan Seafood Wholesale Market in Wuhan, China, as were the first transmission to humans occurred, as all cases of unknown viral pneumonia link back to this Market. SARS-CoV-2 shares 80% and 50% genome sequence identity with previous human coronaviruses, SARS-CoV and MERS-CoV, respectively.

Various institutions rapidly responded to the novel pandemic by developing vaccines aiding in acquiring immunity with exceptional high speed. Currently, the vaccines are unequally distributed. For example in Europe, where the four EMA approved vaccines used in the vaccination program are not equally divided, possibly resulting in a stock shortage of specific vaccines (83). Therefore, combining vaccines would make the vaccination rollout more flexible.

Additionally, the vaccines translate into different efficiency and safety. Whereas the mRNA vaccines result in an protective efficiency of 90-95%, the adeno vector vaccines display a lower efficiency of an average 70%. Furthermore, the vector vaccine of Oxford/AstraZeneca possibly links to rare blood clots with low blood platelets in certain age groups (84). By combining the different vaccines, the efficiency and safety profiles possibly can reach a plateau, with a high percentage of efficiency and reduced risks of adverse events.

The idea of prime-boosting individuals with heterologous vaccines is based on the underlying thought to induce both a cellular and humoral immune response, since specific traditional vaccines are unable to evoke an effective T cell response. Previous studies demonstrated the ability to increase the robustness of immune responses when the prime-boost regime is carried out with heterologous vaccines. Therefore, this strategy is applied in vaccine regimes against various infectious diseases for which requiring immunity poses a challenge.

A phase 2 trial reported preliminary results of boosting individuals who previously received a single dose of Oxford/AstraZeneca with the Pfizer/BioNTech vaccine (80). The results demonstrate an increase in the anti-spike antibodies and neutralizing antibodies. Furthermore, a 4-fold increase in in cellular immunity was detected. The levels of neutralizing antibody titres correlate with protective effects and long-lived immunity, indicating the importance of achieving maximum neutralizing antibody titres (85). Notably, the phase 2 trial does not compare the result of the heterologous to homologous regimes using only adeno vector vaccines or mRNA COVID vaccines.

A randomized controlled trial published results concerning the immunogenicity of the heterologous regime. In this trial, both heterologous regimes were compared to both homologous regimes. Only four serious adverse events were reported, however none of which linked to the vaccination. Therefore, the heterologous vaccine schedule appears to be a safe alternative.

In this study, the homologous Pfizer/BioNTech schedule resulted in the highest amount of anti-spike IgG. Therefore, this study indicates that combining the different vaccines does not results in an increased levels of antibodies. However, the heterologous regimes both still induced considerably high levels of anti-spike IgG. Although the homologous Pfizer/BioNTech schedule resulted in the highest level of anti-spike IgG, both heterologous regimes resulted in higher levels of anti-spike IgG as compared to the homologous Oxford/AstraZeneca.

Beside the humoral immune response is the observed T cell response, which is remarkable. The Oxford/AstraZeneca-Pfizer/BioNTech heterologous schedule demonstrated a 2-fold increase in T cell response compared to the homologous regimes. Although the Pfizer/BioNTech-Oxford/AstraZeneca regime does not demonstrate the same steep increase, the T cell response is still considerably higher than observed in both the homologous regimes. In addition to the humoral response, T cells are presumably required in a strong, effective immunity to SARS-CoV-2 and the alarming new variants (107). Therefore, although the humoral responses of the homologous regimes were not superior to that induced by the homologous Pfizer/BioNTech regime, the increased T cell response may elicit a more robust and broad immune response, resulting in effective immunity to SARS-CoV2.

Even though antibody levels are highly predictive, they do not necessarily translate to protective immunity (86). The randomized controlled trial does give an indication only about the immunogenicity. This study must therefore form a base for future research. Additional trials, using homologous schedules for comparison, need to validate the clinical effectiveness and safety of the heterologous schedules. After the results of the trial were published, various European government institutions incorporated the heterologous Oxford/AstraZeneca followed by Pfizer/BioNTech regime into the vaccination program. Currently, insufficient data about combining Oxford/AstraZeneca with the other mRNA COVID vaccine of Moderna is present. Therefore, it would be interesting for future studies to evaluate the safety and efficiency of combining Moderna with Oxford/AstraZeneca.

Since the heterologous schedules appear to be safe and yield an sufficient immune response, vaccination rollout can become more flexible. By increasing the vaccination speed, the spreading of the virus becomes less and is therefore less likely to mutate into a more virulence mutant. The current studies indicate beneficial effects from the heterologous regimes, as a robust immune response is demonstrated, both humoral and cellular. Therefore, I would recommend to keep the heterologous regime, whereby individuals are primed with Oxford/AstraZeneca and thereafter boosted with Pfizer/BioNTech, incorporated into the vaccination program.

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