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COMBATING SARS-COV-2: A COMPARISON BETWEEN MRNA VACCINES AND KILLED WHOLE CELL VACCINES

ROY S[1*], CHAKRABARTY S[2], PAL R[2] AND CHOUDHURY L[3]

1: Assistant Professor, Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata-700 016, West Bengal, India

2: 5th Year Integrated M. Sc. (5 year integrated) students, Post-Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata-700 016, West Bengal, India

3: State-Aided College Teacher, Department of Microbiology, Sarsuna College (under Calcutta University), 4/HB/A, Ho-Chi-Minh Sarani, Sarsuna Upanagari, Kolkata – 700061, West Bengal, India

***Corresponding Author: Dr. Souvik Roy: E Mail: souvikroybiotech@sxccal.edu**

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ABSTRACT

The ongoing pandemic of COVID-19 has created havoc in the world. With over 190 million COVID-19 cases worldwide, scientists are racing towards developing vaccines for Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the etiologic agent of COVID-19. Vaccine elicits both cell-mediated and humoral branches of immunity by the production of effector cells and memory cells, thereby stimulating a strong adaptive response in the vaccinee. Among well over 120 vaccine candidates being scrutinized around the world, some have proven to be very potent in combating SARS-CoV-2. This review presents the outcomes from the pre-clinical studies of mRNA vaccines and killed whole cell vaccines (KWCVs) on suitable animal models, as well as those of Clinical Phase I/II human trials, thereby evaluating their comparative efficacies, emphasizing on the adaptive immune responses developed upon immunization with each of them.

Keywords: COVID-19; killed whole cell vaccines (KWCVs); mRNA vaccines; SARS-CoV-2

1. INTRODUCTION

The world was hit by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) in the month of 2019 December, which led to the global outbreak of a respiratory disease called COVID-19 **[1]**. With well over 120 vaccine candidates that came out in the first 6 months of the outbreak, the mRNA 1273 vaccine manufactured by Moderna and the two dose BNT162b1 mRNA vaccine manufactured by Pfizer-BioNTech became two of the most prominent candidates **[2]**. This technique uses a nucleoside-modified messenger RNA (mRNA)–based vaccine that is encapsulated by lipid nanoparticle, encoding for the SARS-CoV-2 spike (S) glycoprotein Receptor Binding Domain (RBD) which is stabilised it its prefusion conformations so as to prevent rearrangement in the structure of the glycoprotein **[3]**. SARS-CoV-2 S glycoprotein RBD on the viral envelope binds to the cellular angiotensin-converting enzyme-2 (ACE2) receptor, which initiates a cascade of signalling events, eventually leading to the viral membrane fusion with the susceptible host cell membrane, leading to entry into the host cell **[4]**.

Killed whole cell vaccine (KWCV) is one of the oldest techniques which had given effective protection against cholera and many other contagious diseases **[5, 6]**. Various studies have been performed with these vaccines. No adverse effects were seen when the KWCVs against enterotoxicogenic *E. coli* (ETEC) were administered orally in human subjects **[7]**. Similarly, enterotoxicogenic *E. coli* vaccine (EV) expressing colonization factors which are responsible for causing diarrhea are safe options, as it was found not to hamper the normal microflora of the gastrointestinal (GI) tract of the vaccinee **[6, 8, 9]**. The KWCVs against ETEC responsible for diarrhea were immunized in human volunteers in a study, showing no adverse effects **[10]**. Similarly in children, the KWCVs against ETEC, when administered with adjuvants, also showed no symptoms and were found to be safe **[11]**. KWCVs against a mutant version of *E. coli* strain, the J5 variant, were also seen to be safe in calves **[12]**. This J5 KWCV for bovine mastitis has already been licensed **[12]**. Besides, the KWCV Fusion Protein (FP) vaccine against SARS-CoV-2 has given significant protection against the viral pathogenic effects. The FP has been selected because it has been found to be extremely conserved among all the coronaviruses, especially the 6 core amino acid residues **[13]**. However, the deletion of the non-essential surface-expressed proteins from the genome, the immunization schedule, route and the use of appropriate adjuvants may elicit a better immune response **[13]**. Additionally, they

are also inexpensive, easier to manufacture and resistant to evolution, and hence may give support to the developing countries where the recent vaccines are not available, either due to high cost or due to inadequate health care infrastructure **[13]**. All the KWCVs applied so far have an excellent safety record, and are attractive immunization options after the COVID-19 pandemic.

Although several potent vaccine candidates have emerged in recent times to combat SARS-CoV-2, this review focuses on the design and comparative efficacies of mRNA vaccines and KWCVs against the same.

2. STRATEGIES FOR DESIGNING VACCINES AGAINST SARS-CoV-2

2.1. mRNA Vaccines

Different vaccine approaches have been worked out to strengthen the host immunity against novel coronaviruses. One such revolutionizing approach makes the use of RNA as a delivery system to enhance stability as well as translatability of the RNA within the cell **[14]**. The mRNA vaccine discards off the risk of insertional mutagenesis, being a non-infectious and non-integrating molecule. The mRNA is formulated into carrier molecules, which enable them to be taken up and expressed in the host cell cytoplasm **[15]**. The mRNA being a minimal genetic vector only contains the genetic element required for the expression of the encoded target protein, and hence it does not trigger anti-vector immunity. Therefore, it can be administered in repeated doses **[16, 17, 18]**. The development and commercial production of the mRNA vaccine is not a time-consuming process, which makes it a very good choice for vaccine technology during, and after a pandemic. The two mRNA vaccines that are permitted for emergency use for SARS-CoV-2 are BNT162b1 and mRNA 1273 vaccines **[2]**.

The mRNA vaccine developed by Pfizer-BioNTech, called the BNT162b1, is a mRNA vaccine that encodes for the SARS-CoV-2 S glycoprotein RBD **[3]**, thereby eliciting an innate immune reaction, causing an increase in the expression of the target antigen. The vaccine is encapsulated into a lipid nanoparticle (LNP) which allows the mRNA to be transfected into the host cell followed by an intramuscular injection **[19]**. Upon injecting the LNP-encapsulated mRNA vaccine into the host, the LNPs are taken up by the cell, and the mRNA is released into the cytosol where it gets translated, leading to the expression of the viral protein **[19]**. The spike protein expressed in the cytosol undergoes degradation, forming smaller peptide fragments **[19]**. These peptide fragments are presented on the cell surface, which generates a T-cell mediated humoral

immune response against SARS-CoV-2 **[19]**.

The mRNA 1273 vaccine that was first developed by Moderna encodes for a stabilized prefusion S glycoprotein of SARS-CoV-2 **[20]**. Coronaviruses are covered with spikes which bind to the proteins on the host cell, and transform themselves to fuse with the latter **[20]**. This fusion needs to be prevented by antibodies for the vaccine to be effective. However, the S glycoproteins are subjected to changes that cause the pre-fusion conformation to shift into a post-fusion conformation **[20]**. It was noted that the addition of two prolines (2P) resulted in the stabilization of the pre-fusion state of the S glycoprotein **[20]**. The 2P mutation was studied in Moderna's Middle East respiratory syndrome (MERS) vaccine, before it was used in the COVID-19 mRNA vaccine **[20]**.

2.2 Killed Whole Cell Vaccines (KWCVs)

Gram-negative bacteria such as *E. coli* use the autotransporters (ATs) to transport proteins from the cytosol to their outer membrane (OM) **[21-24]**. ATs mainly consist of 3 domains: N-terminal signal sequence to direct the protein through inner membrane (IM), C-terminal β-barrel domain which forms a pore-like structure by getting inserted across the OM, and a central passenger domain which transits through the pore, thus making the protein available to the outside environment **[25, 26]**. A systemic set of deletions in the genome of various *E. coli* strains were constructed and these viable, slow-growing, genome-reduced bacterial strains were used to produce KWCVs **[27, 28]**. The SARS-CoV-2 mainly utilises the S protein for entering the host cell. Following proteolytic cleavage, it gets split into S1 and S2 **[29, 30]**. The S1 mainly binds with the receptor, whereas the S2 is a FP which mediates viral-bacterial membrane-membrane fusion to get entry into the cell **[24]**. So, the KWCV can be administered for passive immunization, which will introduce the pathogenic antigen (in this case the FP of SARS-CoV2) in order to elicit an immune response to generate antibodies **[23, 24]**.

3. ANALYSING THE EFFICACY OF VACCINES AGAINST SARS-CoV-2

3.1 mRNA Vaccines

3.1.1 Procedure

3.1.1.1 For BNT162b1 mRNA Vaccine

LNP-encapsulated RNA vaccine candidates, evolved in 'Project Light-speed', the joint BioNTech-Pfizer COVID-19 RNA vaccine development programme, was delved into during the two Phase I/II trails in Germany and the USA **[31]**. The RBD of the SARS-CoV-2 spike protein, a crucial and significant target of neutralising the antibodies, is encoded by BNT162b1 **[31, 32]**. Dosages of 10 μg, 30 μg (prime

and boost doses, 3 weeks apart for both dose-levels) and 100 μg (prime only) were sent for supervision in the placebo-controlled, observer-blinded USA trail **[33]**. There were no reports of detrimental or harmful events **[33]**. Local injection site-reactions, and systemic events resembling that of influenza, were subjected to the related dose, ranging from mild to moderate and transient **[33]**. RBD-binding Immunoglobulin G (IgG) concentrations and SARS-CoV-2 neutralising titres in the sera increased with the level of dose, and increased further after the second dose fourteen days after the booster dose, mean value of the neutralising titres reached 1.9- to 4.6-fold than those observed in a panel of COVID-19 human convalescent sera (HCS) **[33-35]**. This study enhanced and enlarged the former report with the existing data from the German trial, providing a meticulous characterisation of antibody and T-cell immune responses elicited by vaccination with BNT162b1 **[33]**. As many as 60 participants were vaccinated with BNT162b1 in Germany in the time period 23 April 2020 - 22 May 2020, which was set for studying design and drawing analysis **[33]**. For each of the dose-level groups, 12 participants received the first dose on the first day, and a booster dose exactly three weeks later, on day 22 (except for an individual in each of the 10 μg and 50 μg dose- level cohorts who desisted from attendance on grounds not linked to the drug in study), and 12 participants received a 60 μg prime dose on the first day only **[33]**. The populace of the study consisted of healthful males and non-pregnant females, with an age of 37 years (range of 20-56 years) being the mean with identical distribution between both genders **[33]**.

3.1.1.2 For mRNA 1273 Vaccine

Execution of animal experiments took place at US National Institutes of Health Regulations, with abidance by all the rules, and with approbation from the Animal Care and Use Committee (ACUC) of the Vaccine Research Centre, Moderna, and the University of North Carolina **[34-36]**. 6- to 8- week-old female BALB/cJ, C57BL/6J and B6C3F1/J mice were used for examination of immunogenicity **[34-36]**. Mice were inoculated as an act of suffusion intravenously in the similar hind leg for both prime and boost shots **[37]**. The control mice had received phosphate buffered saline (PBS), as it was seen that mRNA formulations being tested does not cause a significant non-specific immunity beyond a few days **[37]**. Mice were infused intramuscularly with Sigma Adjuvant System (SAS) for all SARS-CoV-2 S(2P) protein vaccinations **[37]**. In mice with SARS-CoV-2 S(2P) and alum immunization, the SARS-CoV-2 S(2P) protein and 250 μg alum hydrogel was

injected intramuscularly **[37]**. The basis of attributes set by institutional ACUC was made use of to conclude the sample size for animal experiments **[37]**. This was to ensure that the experiments were not run in a haphazard or blinded fashion **[37]**.

3.1.2 RESULTS

3.1.2.1 For BNT162b1 mRNA Vaccine

3.1.2.1.1 Antibody response

Non-randomised open-label Phase I/II trials, when conducted in healthy adults after vaccination with two doses of 1-50 μg of BNT162b1, generated strong $CD4^+$ and $CD8^+$ T cell mediated antibody responses, with an elevated concentration of IgG against S glycoprotein RBD **[33]**. This increase in concentration was prominently higher than those observed in the serum from the COVID-19 recovered individuals **[33].** Most of the participants that took part in the Phase I/II trials showed an increased T helper type 1 (T_H1) response level with an elevated RBD-specific $CD8^+$ and $CD4^+$ T cell productions **[30]**.

On the 22nd day, 21 days after receiving the priming dose for the 4 different dose-levels (1 μg, 10 μg, 30 μg and 50 μg), there was a distinct dose-dependent increase in the Geometric Mean Concentration (GMC) of RBD binding IgG, which went from 265 U ml^{-1} for the 1 μg dose-level cohort to 1,672U ml^{-1} for the 50 μg dose-level cohort **(Table 1) [33]**. On the 29th day, 7 days after the booster dose, the GMC of the IgG showed a similar increase in all the dose-levels, ranging from 2015 U ml^{-1} to 25,006 U ml^{-1} **[33]**. The trend continued till the 43rd day, 21 days after the booster dose, when the IgG GMC was observed to be 3920 U ml^{-1} for the 1 μg dose-level cohort to 18,289 U ml^{-1} in 50 μg dose-level cohort for the vaccinated individuals **[33]**. This level was significantly higher than the IgG GMC of 602 U ml^{-1} taken from 38 patients, 14 days after they were diagnosed with SARS-CoV-2, using Polymerase Chain Reaction (PCR) **[33]**. For the 60 μg dose-level cohort, having received only the priming dose, the IgG GMC was observed to be 755 U ml^{-1} on the 43rd day, which further stresses on the necessity of a booster dose to increase the concentration of the IgG antibody **[33]**.

3.1.2.1.2 T cell response

The vaccine-induced $CD4^+$ and $CD8^+$ T cell responses were monitored using direct *ex vivo* IFN-γ enzyme-linked immunosorbent spot (ELISpot) assay with the help of peripheral blood mononuclear cells (PBMCs) collected from 51 participants across all the dose-level cohorts from 1 μg to 60 μg **(Table 2; Figure 1)**. For this assay, the $CD4^+$ and $CD8^+$ T cells were triggered overnight with overlapping peptides which represented the full-length sequence of the S glycoprotein RBD encoded by the vaccine **[33]**. 40 out of the 42 participants who had received the

priming dose of the vaccination (from 1 μg to 50 μg dose-levels) showed RBD-specific $CD4^+$ T cell responses, which accounted for 95.2% of the participants **[33]**. The 2 participants not showing a $CD4^+$ T cell response had received a 1 μg dose-level **[33]**. The 60 μg dose-level cohort that did not receive the booster dose showed a decreased immunogenicity and rate of response than the other sets of doses which highlighted the significance of the booster dose of the vaccination **[33]**. Only 5 out of the 9 participants (55.5%) showed a RBD-specific $CD4^+$ T cell response in case of the 60 μg dose-level cohort **[33]**. This magnitude of the $CD4^+$ T cell response had a clear correlation with the study performed with the RBD-binding IgG concentration **[33]**.

A similar case was observed for participants who mounted a $CD8^+$ T cell response **(Table 2; Figure 1) [33]**. 32 out of the 42 participants (76.2%) produced a surge in the $CD8^+$ T cell response, with an identical decline in the immunogenicity and response in case of individuals who were administered the 60 μg dose of the vaccine **[33]**. Participants with RBD specific $CD8^+$ T cell response went down to 44.4% (4 out of the 9 participants) for the 60 μg dose-level cohort **[33]**. All the findings strongly suggested that individuals immunized with the BNT162b1 elicited a prominent $CD4^+$ and $CD8^+$ T cell response in majority of the participants, as well as a T_H1 polarization of the helper response.

3.1.2.2 For mRNA 1273 Vaccine

3.1.2.2.1 Antibody response

The immunogenicity was measured in 6- to 8- weeks old female BALB/cJ, C57BL/6J and B6C3F1/J mice receiving intramuscular immunization, on the 1st and 22nd day, with three different dose-levels (0.01, 0.1 and 1 μg) of the mRNA 1273 vaccine **(Figure 2) [38]**. The vaccine resulted in production of dose-dependent S glycoprotein-binding antibodies after the first dose and booster dose in all the mouse strains that were used for the experiment **[38]**. A strong pseudovirus-neutralizing activity was also seen to be stimulated on immunization with the 1 μg dose-level of mRNA 1273 vaccine which increased gradually between the 2nd and 4th week by the mRNA 1273 vaccine **[38]**. This study demonstrated that an effective immunogenic response is triggered on mRNA 1273 administration **[38]**.

3.1.2.2.2 T cell response

There have been challenges identical to that of Vaccine-Associated Enhanced Respiratory Disease (VAERD) observed in numerous animal models when experiments were conducted with whole inactivated vaccines for SARS-CoV-2 **[38]**. This disease has been linked with T helper 2 (T_H2) cell-based immune responses in young children **[38]**. This led to further

investigation and research for studies on the balance between the T_H1 and T_H2 cell responses in mice after immunization.

On eliciting the S-specific immunoglobulins, IgG2a and IgG2c, which represent the T_H1 response, and IgG1 which represents the T_H2 response by the mRNA 1273 vaccine and SARS-CoV-2 S(SP) protein using Toll-like Receptor (TLR)-4 agonist SAS, the responses were compared and analysed **(Figure 3a-c) [37, 38]**. Splenocytes where isolated from mice 7 weeks after they were administered with the booster dose, and they were re-stimulated with a pool of overlapping peptides from the S protein of SARS-CoV-2 in the presence of a transport inhibitor **[38]**. mRNA 1273 triggered $CD4^+$ T cells, when re-stimulated with S1 or S2 peptide, showed a dominant T_H1 response with an elevated level of T_H1 cytokine prototype [interferon (IFN)-γ] **[38]**. A strong $CD8^+$ T cell response to the S1 peptide was obtained upon immunization with mRNA 1273 in mice, but was absent in case of the SARS-CoV-2 S(2P)-administered mice **[38]**. At the same time, re-stimulating SARS-CoV-2 S(2P) protein using the TLR4 agonist SAS with alum adjuvant, elicited a T_H2 biased-response, indicating that mRNA vaccination prevents T_H2-based immune response that is associated with VAERD **[38]**.

Table 1: IgG concentration in BNT162b1 immunized participants

Dose-level cohorts	IgG (U ml^{-1})			
	Day 8	Day 23	Day 29	Day 43
1 µg	3	265	2,015	3,920
10 µg	1	826	9,107	6,707
30 µg	2	1,273	17,051	12,431
50 µg	1	1,672	25,006	18,289

Table 2: RBD-specific $CD4^+$ and $CD8^+$ T cell response in BNT162b1 immunized participants

All dose-level cohorts	Participants (%) showing Immunogenicity	
	RBD-specific $CD4^+$ T cell response	RBD-specific $CD8^+$ T cell response
1 µg	81.8	72.7
10 µg	100	81.8
30 µg	100	80
50 µg	100	70
60 µg	55.5	44.4

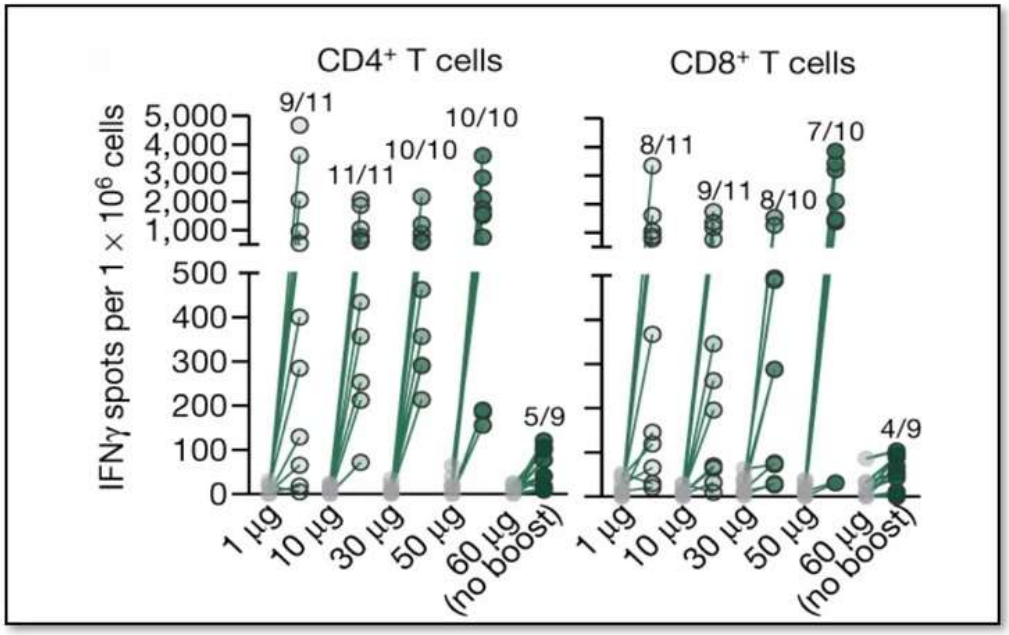

Figure 1: RBD-specific CD4$^+$ and CD8$^+$ T cell responses for all dose cohorts (Adapted from "COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses" by Sahin, U., Muik, A., Derhovanessian, E. *et al.*, 2020, *Nature* 586, 594–599. 2021 by Clinical and Experimental Vaccine Research.)

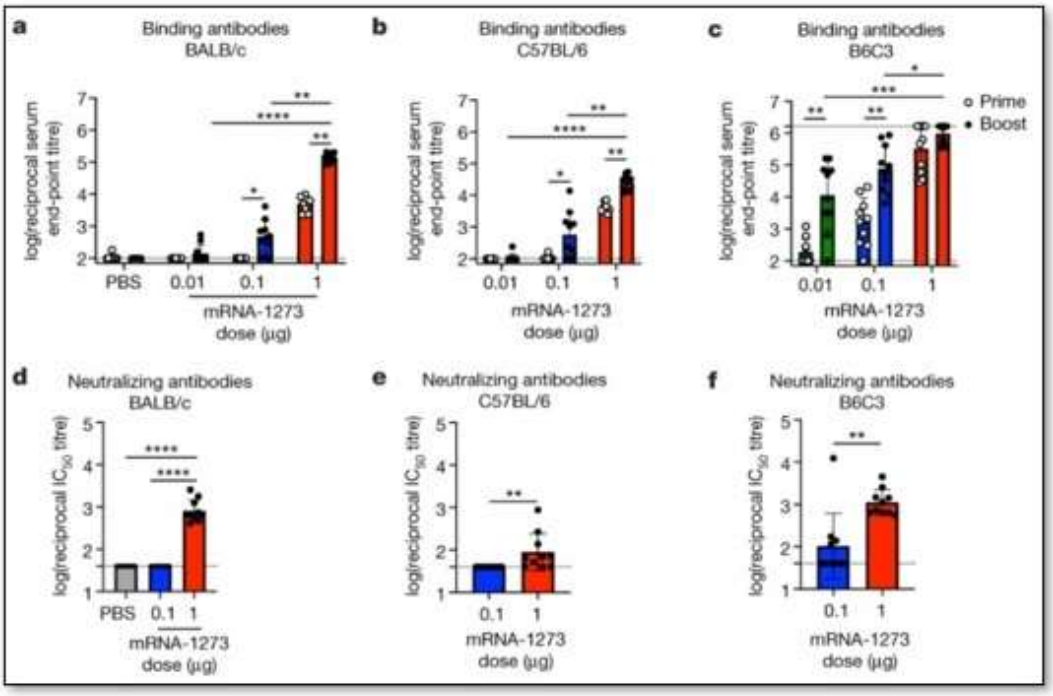

Figure 2: Specific S binding and pseudovirus-neutralizing antibody responses in three different mouse strains immunized with mRNA-1273 Vaccine: a-f: BALB/cJ (a, d), C57BL/6J (b, e) and B6C3F1/J (c, f) mice (10 for each strain) were immunized on 1st and 22nd day with three different doses 0.01 µg (shown in green), 0.1 µg (shown in blue) and 1 µg (shown in red) of mRNA 1273. PBS (shown in grey) was injected in the control BALB/cJ mice. At the end of 2 weeks after prime (denoted by unshaded circles) dose and 2 weeks after the booster dose (denoted by shaded circles), the sera were collected and evaluated for the presence of IgG antibody specific for the SARS-CoV-2 spike protein using the technique of enzyme-linked immunosorbent (ELISA) assay (Adapted from "SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness" by Corbett, K.S., Edwards, D.K., Leist, S.R. *et al.*, 2020, *Nature* 586, 567–571. 2021 by Clinical and Experimental Vaccine Research).

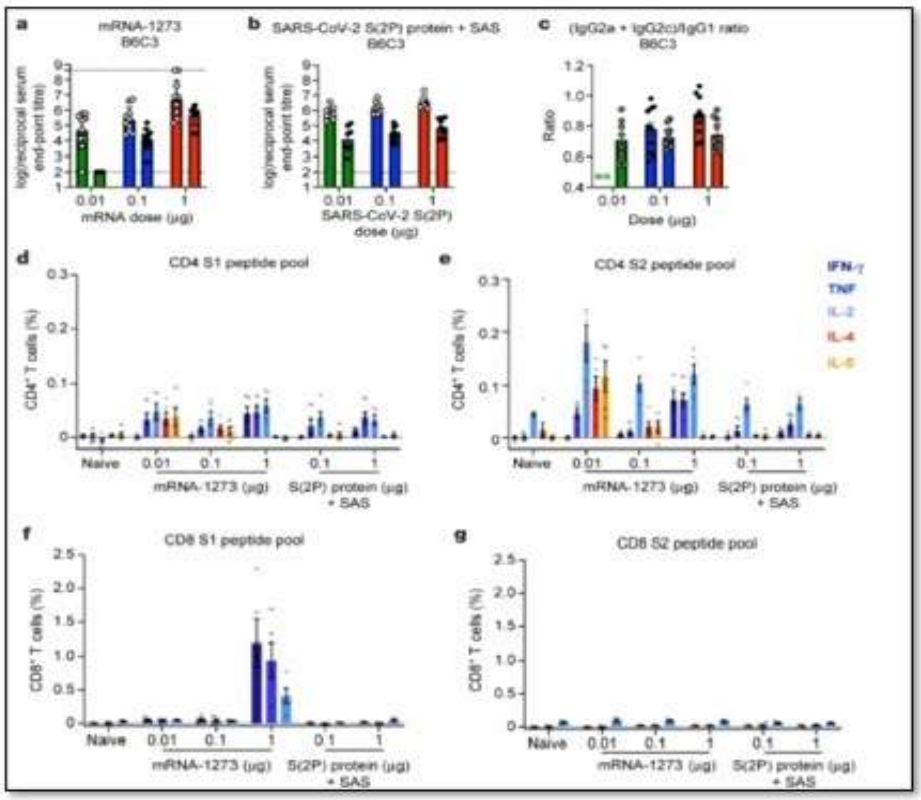

Figure 3: S-specific T_H1-biased T cell responses elicited by on immunization with mRNA 1273 Vaccine: a-g: B6C3F1/J mice were administered mRNA 1273 vaccine or SARS-CoV-2 S(2P) protein with SAS adjuvant on the 1st and 22nd day with 0.01 (green), 0.1 (blue) or 1 µg (red) dose-levels; a-c, Sera were collected two weeks after the prime dose (denoted with unshaded circles) and two weeks post the booster dose (denoted with shaded circles) and they were evaluated for the IgG1, and IgG2a and IgG2c antibody specific for the SARS-CoV-2 S protein; d, showing the percentage of the $CD4^+$ T cells response to the S1 peptide pool; e, percentage of $CD4^+$ T cell response to the S2 peptide pool; f, percentage of $CD8^+$ T cell response to the S1 peptide pool; g, percentage of $CD8^+$ T cell response to the S2 peptide pool (Adapted from "COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses" by Sahin, U., Muik, A., Derhovanessian, E. *et al.*, 2020, *Nature* 586, 594–599. 2021 by Clinical and Experimental Vaccine Research).

3.2 Killed Whole Cell Vaccines (KWCVs)

3.2.1 Procedure

To test for the antibody binding in genome-deleted bacterial cells, a specific dilution of bacterial cells was added to 96-well microtiter plate, washed, and incubated with anti-HA primary antibody and rabbit sera anti-SARS-CoV-2 FP antibody **[39, 40]**. The samples were washed, stained and analysed using FACS Calibour flow cytometer **[40]**. Pigs are quite similar to humans in anatomy, physiology and genetic make-up. As there is a stretch of 13-amino acid residues that is identical between the Porcine Epidemic Diarrhea Virus (PEDV) FP and SARS-CoV-2 FP, it was possible to test the efficacy of SARS-CoV-2 FP vaccine using the PEDV vaccine model **[40]**. A total of 21 piglets under study were administered with the killed whole genome-reduced vaccines expressing SARS-Cov2 FP or the PEDV FP, keeping killed whole bacterial cells as control **[40]**.

The booster dose was given 21 days post-vaccination (dpv) and the serum samples from these pigs were collected at 1-, 3-, 5- and 6-weeks post vaccination (wpv) and before vaccination as well **[40]**. The pigs were challenged with the PEDV via oral inoculation on 35 dpv **[40]**. Finally, 7 days post challenge (dpc) the pigs were sacrificed, and intestinal tissue samples were collected from them for detecting and quantifying the PEDV RNA load **[40]**. The total RNA was isolated from homogenized intestinal tissue or sample using TRIzol LS Reagent and quantified using one step qRT-PCR **[40]**.

For the ELISA test used for detecting anti-PEDV FP and anti-SARS-CoV-2 FP antibody, the 96-well microtiter plate was coated with BSA-conjugated peptides of SARS-Cov-2 FP and PEDV FP, washed and blocked with a suitable buffer, after which a suitable dilution of the serum sample was added to these plates **[40]**. These plates were incubated with a suitable dilution of peroxidase-conjugated rabbit anti-pig IgG **[40]**. The serum from pigs which were not challenged with PEDV was used as negative control, while hyperimmune pigs' serum was used as positive control **[40]**. Finally, 3,3′,5,5′-tetramethylbenzidine (TMB) solution was added, the reaction was stopped by adding sulphuric acid (H_2SO_4), and absorbance reading was taken at 450 nm (OD_{450}) **[40]**.

For the detection of IFN-γ, the 96-well microtiter plate was pre-coated with IFN-γ specific antibody, and to this, two-fold serially diluted IFN-γ samples and undiluted pig serum samples were added and incubated, which was followed by the addition of biotin-conjugated antibody to the plates **[40]**. The plates were then washed and incubated with streptavidin-horse radish peroxidase (HRP), and the colour was obtained by using TMB substrate, followed by adding a stop solution and taking the absorbance at 450 nm (OD_{450}) **[40]**.

3.2.2 RESULTS

3.2.2.1 Antibody response in pigs after vaccination

The pigs were immunized with the killed whole genome reduced vaccines expressing the SARS-Cov-2 FP, PEDV FP or control bacteria which do not express any FP **[40]**. They were given booster dose on the 21st day and challenged with PEDV on the 35th day, while the blood samples were collected daily **(Figure 4) [40]**. ELISA was performed for determination of antibodies which could recognize the FP expressed on the surface of the bacteria. It was found that vaccination with either the SARS-CoV-2 FP or PEDV FP vaccines did not elicit a potent anti-FP response by the 5th week **[40]**. But 2 weeks after giving a booster dose, there was a small, yet significant, increase in anti-PEDV FP antibody in the

pigs which have been vaccinated with the SARS-CoV-2 FP vaccine **[40]**. However, there was no significant increase in anti SARS-CoV-2 FP antibody in those pigs which have been immunized with the SARS-CoV-2 FP itself **[40]**. A high amnestic response was obtained against both PEDV FP and SARS-CoV-2 FP on the day of necropsy **[40]**. From the results obtained, it could be concluded that there was a significantly enhanced response against both SARS-CoV-2 FP and PEDV-FP, which was higher than the pigs immunized with the control vaccine showing no FP on its outer surface **[40]**.

3.2.2.2 IFN-γ response in vaccinated pigs

The IFN-γ response was examined between vaccinated and control groups regularly. A prominent difference was noted 5 weeks post vaccination, and 1 week post challenge **[40]**. From the results it was also found that the serum IFN-γ levels significantly increased 2 weeks after the vaccine booster dose (5 wpv), and 1 week post challenge (1 wpc) in the vaccinated groups, as compared to the control group **(Figure 5) [40]**. Initially at 1 wpv and 3 wpv, the IFN-γ levels were identical, but the level increased at 5 wpv (2 weeks after the booster dose) in vaccinated groups **[40]**. So, this indicated that the T cells were activated by the vaccine prime dose, which was further amplified by the booster dose, suggesting that the vaccines were potentiating an IFN-γ response in immunized animals **[40]**.

3.2.2.3 Viral RNA load in intestinal tissue of vaccinated pigs

The small and large intestinal contents (jejunum, colon, cecum) were collected during necropsy, which is 7 dpc to quantify the viral RNA load in each sample by qRT-PCR **[40]**. For jejunum tissue, a significant difference in PEDV RNA loads was noted in the pigs vaccinated with PEDV, compared to the control set which was vaccinated only with KWC genome-reduced vaccine **[40]**. The difference was not so prominent in the colon, cecum tissue and small intestinal contents **[40]**. SARS-Cov-2 FP vaccinated pigs also showed lower, but not significant viral load compared to control pigs **(Figure 6) [40]**. Hence, the vaccine resulted in a reduction of viral RNA load in the jejunum tissue of pigs which has been challenged with PEDV **[40]**.

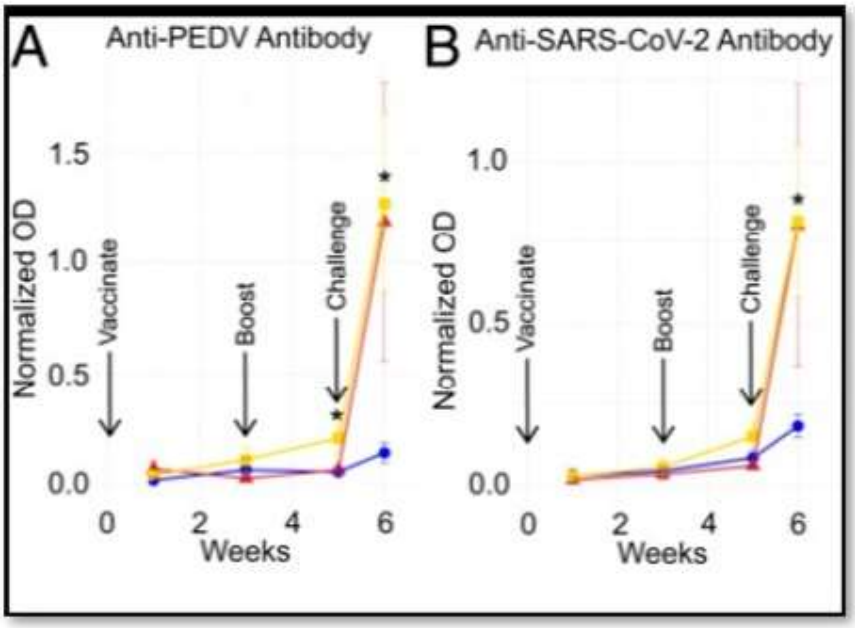

Figure 4: Humoral immune response in pig models on immunization with KWC genome reduced vaccines: a-b, Immunized pigs showing humoral immune responses. The pigs were vaccinated with SARS-CoV-2 FP (yellow), PEDV FP (red) genome-reduced KWCVs and control KWCV showing no FP (blue). The blood samples were collected daily for examination, booster dose was given 21 days after the first dose and the pigs were challenged with PEDV on the 35th day. The amount of anti-PEDV antibody and anti-SARS-CoV-2 antibody present in the sera of the pigs were determined by performing ELISA (Adapted from "Killed whole genome-reduced bacteria surface-expressed coronavirus fusion peptide vaccines protect against disease in a porcine model" by Maeda, D. L. N. F., Tian, D., Yu, H., Dar, N., Rajasekaran, V., Meng, S. *et.al.*, 2021, *Proc Natl Acad Sci* U S A, 118(18). 2021 by Clinical and Experimental Vaccine Research).

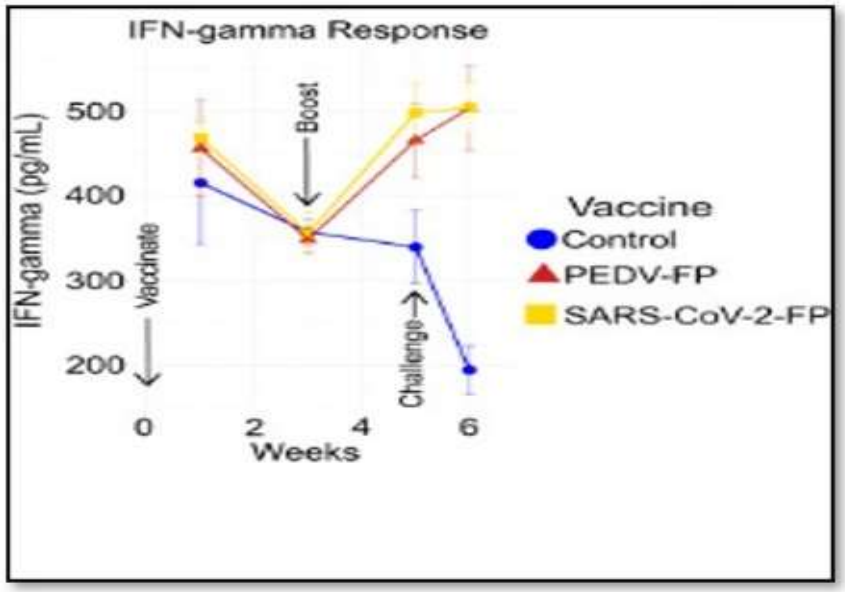

Figure 5: IFN-γ response in the serum of immunized pig models. The serum samples from control and vaccinated groups of pigs models against PEDV FP KWCV (red), SARS-COV-2 FP KWCV (yellow) and control KWCV with no FP (blue) were obtained are the IFN-γ response was determined using ELISA (Adapted from "Killed whole genome-reduced bacteria surface-expressed coronavirus fusion peptide vaccines protect against disease in a porcine model" by Maeda, D. L. N. F., Tian, D., Yu, H., Dar, N., Rajasekaran, V., Meng, S. *et.al.*, 2021, *Proc Natl Acad Sci* U S A, 118(18). 2021 by Clinical and Experimental Vaccine Research).

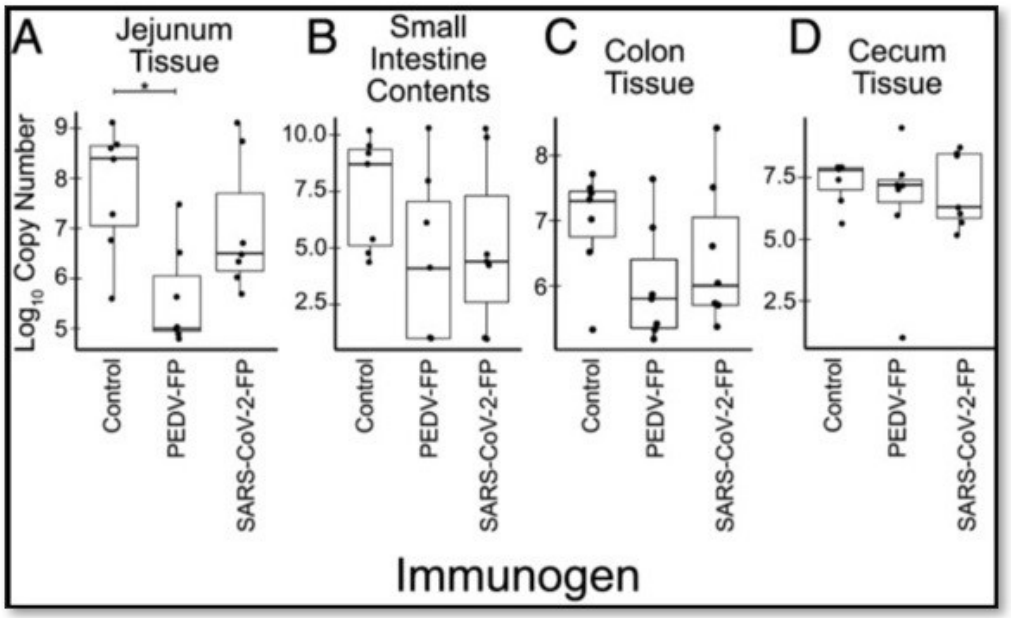

Figure 6: Viral RNA load in the small intestine of immunized pigs. On the day of necropsy the intestinal tissue contents: a, duodenum; b, small intestinal contents; c, colon tissue; d, cecum tissue were collected and the amount of PEDV RNA present in these samples were detected by performing qRT-PCR (Adapted from "Killed whole genome-reduced bacteria surface-expressed coronavirus fusion peptide vaccines protect against disease in a porcine model" by Maeda, D. L. N. F., Tian, D., Yu, H., Dar, N., Rajasekaran, V., Meng, S. *et.al.*, 2021, *Proc Natl Acad Sci* U S A, 118(18). 2021 by Clinical and Experimental Vaccine Research.)

4. CONCLUSION

Vaccine technology has made an overwhelming progress in the recent times. The development of vaccines usually takes several years before it is given the license to be used on human population. However, the phases I and II clinical trials of the mRNA vaccines are conducted within a couple of months, which is a big step in the right direction. The safety, efficacy and cost of the vaccine candidates are of a major public concern. This review tries to give a comprehensive synopsis of the comparative analysis of mRNA vaccines and killed whole cell vaccines, outlining the designing strategies and summarizing the relative efficacies of both of these. The data and results given in this review indicates that both of these potent vaccine candidates are quite promising in eliciting a safe and an effective adaptive immune response, thus hoping to revolutionize the science behind vaccine development against COVID-19 in years to come.

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7. CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest.

8. REFERENCES

[1] Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J Clin Med 2020;9:1225-1225.

[2] Safety and Immunogenicity Study of 2019-nCoV Vaccine (mRNA-1273) for Prophylaxis of SARS-CoV-2 Infection (COVID-19) - Full Text View – ClinicalTrials.gov. (2020). ClinicalTrial.Gov. https://clinicaltrials.gov/ct2/show/NCT04283461.

[3] Mulligan, M.J., Lyke, K.E., Kitchin, N. *et al.* Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 586**,** 589–593 (2020). https://doi.org/10.1038/s41586-020-2639-4.

[4] Lan, J., Ge, J., Yu, J. et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581, 215–220 (2020). https://doi.org/10.1038/s41586-020-2180-5.

[5] Q. Bi et al.; Oral Cholera Vaccine Working Group of The Global Task Force on Cholera Control, Protection against cholera from killed whole-cell oral cholera vaccines: A systematic review and meta-analysis. Lancet Infect. Dis. 17, 1080–1088 (2017).

[6] G. Frankel, E. Z. Ron B. Nesta, M. Pizza, "Vaccines against Escherichia coli" in Escherichia coli, a Versatile Pathogen, G. Frankel, E. Z. Ron, Eds. (Springer International Publishing, Cham, 2018), pp. 213–242.

[7] A. Gohar, N. F. Abdeltawab, A. Fahmy, M. A. Amin, Development of safe, effective and immunogenic vaccine candidate for diarrheagenic Escherichia coli main pathotypes in a mouse model. BMC Res. Notes 9, 80 (2016).

[8] J. Holmgren et al., Development and preclinical evaluation of safety and immunogenicity of an oral ETEC vaccine containing inactivated E. coli bacteria overexpressing colonization factors CFA/I, CS3, CS5 and CS6 combined with a hybrid LT/CT B subunit antigen, administered alone and together with dmLT adjuvant. Vaccine 31, 2457–2464 (2013).

[9] M. P. Hays, A. C. Ericsson, Y. Yang, P. R. Hardwidge, vaccinating with conserved Escherichia coli antigens does not alter the mouse intestinal microbiome. BMC Res. Notes 9, 401 (2016).

[10] D. G. Evans, D. J. Evans Jr, A. R. Opekun, D. Y. Graham, Non-replicating oral whole cell vaccine protective against enterotoxigenic Escherichia coli (ETEC) diarrhea: Stimulation of anti-CFA (CFA/I) and anti-enterotoxin (anti-LT) intestinal IgA and protection against challenge with ETEC belonging to heterologous serotypes. FEMS Microbiol. Immunol. 1, 117–125 (1988).

[11] S. J. Savarino et al.; PRIDE Study Group, Oral, inactivated, whole cell enterotoxigenic Escherichia coli plus cholera toxin B subunit vaccine: Results of the initial evaluation in children. J. Infect. Dis. 179, 107–114 (1999).

[12] H. A. Deluyker, P. Rossitto, S. N. Van Oye, J. S. Cullor, Efficacy of an Escherichia coli J-5 mutant strain bacterin in the protection of calves from endotoxin disease caused by subcutaneous challenge with endotoxins from Escherichia coli. Vaccine 23, 709–717 (2004).

[13] Cohen, J. (2020). Vaccine wagers on coronavirus surface protein pay off. Science, 370(6519), 894–895. https://doi.org/10.1126/science.370.6519.894.

[14] Florindo, H.F., Kleiner, R., Vaskovich-Koubi, D. et al. Immune-mediated approaches against COVID-19. Nat. Nanotechnol. 15, 630–645 (2020). https://doi.org/10.1038/s41565-020-0732-3.

[15] Kormann MSD, Hasenpusch G, Aneja MK, Nica G, Flemmer AW, Herber-Jonat S, et al. Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. Nat Biotechnol. 2011;29:154–7. doi: 10.1038/nbt.1733.

[16] Conry RM, LoBuglio AF, Wright M, Sumerel L, Pike MJ, Johanning F, et al. Characterization of a messenger RNA polynucleotide vaccine vector. Cancer Res. 1995;55:1397–400.

[17] Jäschke A, Helm M. RNA sex. Chem Biol. 2003;10:1148–50. doi: 10.1016/j.chembiol.2003.12.003.

[18] Chetverin AB. Replicable and recombinogenic RNAs. FEBS Lett. 2004;567:35–41. doi: 10.1016/j.febslet.2004.03.066.

[19] Walsh EE, Frenck R, Falsey AR, et al. RNA-Based COVID-19 Vaccine BNT162b2 Selected for a Pivotal Efficacy Study. medRxiv 2020.

[20] Thompson, Mark & Burgess, Jefferey &Naleway, Allison & Tyner, Harmony & Yoon, Sarang & Meece, Jennifer & Olsho, Lauren & Caban-Martinez, Alberto & Fowlkes, Ashley & Lutrick, Karen & Groom, Holly & Dunnigan, Kayan & Odean, Marilyn & Hegmann, Kurt & Stefanski, Elisha & Edwards, Laura & Schaefer-Solle, Natasha & Grant, Lauren & Ellingson, Katherine & Gaglani, Manjusha. (2021). Prevention and Attenuation of COVID-19 by BNT162b2 and mRNA-1273 Vaccines. 10.1101/2021.06.01.21257987.

[21] Walsh EE, Frenck RW, Jr., Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020 Oct 14.

[22] Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. MRNA Vaccines—A New Era in Vaccinology. Nat. Rev. Drug Discov. 2018, 17, 261–279.

[23] E. van Bloois, R. T. Winter, H. Kolmar, M. W. Fraaije, Decorating microbes: Surface

display of proteins on Escherichia coli. Trends Biotechnol. 29, 79–86 (2011).

[24] J. Jose, T. F. Meyer, The autodisplay story, from discovery to biotechnical and biomedical applications. Microbiol. Mol. Biol. Rev. 71, 600–619 (2007).

[25] Jong, W. S., Soprova, Z., de Punder, K., ten Hagen-Jongman, C. M., Wagner, S., Wickström, D., de Gier, J. W., Andersen, P., van der Wel, N. N., & Luirink, J. (2012). A structurally informed autotransporter platform for efficient heterologous protein secretion and display. Microbial Cell Factories, 11(1), 85. https://doi.org/10.1186/1475-2859-11-85.

[26] Kjærgaard, K., Hasman, H., Schembri, M. A., & Klemm, P. (2002b). Antigen 43-Mediated Autotransporter Display, a Versatile Bacterial Cell Surface Presentation System. Journal of Bacteriology, 184(15), 4197–4204. https://doi.org/10.1128/jb.184.15.4197-4204.2002.

[27] J. Kato, M. Hashimoto, Construction of consecutive deletions of the Escherichia coli chromosome. Mol. Syst. Biol. 3, 132 (2007).

[28] M. Hashimoto et al. Cell size and nucleoid organization of engineered Escherichia coli cells with a reduced genome. Mol. Microbiol. 55, 137–149 (2005).

[29] Lai, A. L., Millet, J. K., Daniel, S., Freed, J. H., & Whittaker, G. R. (2017). The SARS-CoV Fusion Peptide Forms an Extended Bipartite Fusion Platform that Perturbs Membrane Order in a Calcium-Dependent Manner. Journal of Molecular Biology, 429(24), 3875–3892. https://doi.org/10.1016/j.jmb.2017.10.017.

[30] Tang, T., Bidon, M., Jaimes, J. A., Whittaker, G. R., & Daniel, S. (2020). Coronavirus membrane fusion mechanism offers a potential target for antiviral development. Antiviral Research, 178, 104792. https://doi.org/10.1016/j.antiviral.2020.104792.

[31] Tai, W. et al. A recombinant receptor-binding domain of MERS-CoV in trimeric form protects human dipeptidyl peptidase 4 (hDPP4) transgenic mice from MERS-CoV infection. Virology 499, 375–382 (2016).

[32] Karikó, K. et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol. Ther. 16, 1833–1840 (2008).

[33] Sahin, U., Muik, A., Derhovanessian, E. et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 586, 594–599 (2020). https://doi.org/10.1038/s41586-020-2814-7.

[34] John, S. et al. multi-antigenic human cytomegalovirus mRNA vaccines that elicit potent humoral and cell-mediated immunity. Vaccine 36, 1689–1699 (2018).

[35] Bahl, K. et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. Mol. Ther. 25, 1316–1327 (2017).

[36] Vogel, A. B. et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. Mol. Ther. 26, 446–455 (2018).

[37] Dinnon, K.H., Leist, S.R., Schäfer, A. *et al.* A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature 586, 560–566 (2020). https://doi.org/10.1038/s41586-020-2708-8.

[38] Corbett, K.S., Edwards, D.K., Leist, S.R. et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 586, 567–571 (2020). https://doi.org/10.1038/s41586-020-2622-0.

[39] Deming, D. et al. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. PLoS Med 3, E525 (2006).

[40] Maeda, D. L. N. F. (2021, May 4). Killed whole-genome reduced-bacteria surface-expressed coronavirus fusion peptide vaccines protect against disease in a porcine model. PNAS. https://www.pnas.org/content/118/18/e2025622118.