RESEARCH ARTICLE

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Enhanced Heterologous Immuno-Boost (EHIB) Covid-19 Vaccines: a NOVEL CONCEPT

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ABSTRACT: Heterologous immunization with Covid-19 Vaccines may induce a superior immune response. There are 5 different platforms for Covid-19 vaccines and about 16 different vaccines developed. All these vaccines induce an immune response, some have a better humoral response and lesser cellular response while others have better cellular response than the humoral response. Our concerns about this issue; there are SARS-CoV2 variants, and some vaccines have reduced efficacy against infection due to these variants. Secondly the strength and the type of the immune response. Thirdly length of the time that humoral response lasts and when one must take the booster. To address the above concerns, we recommend having heterologous vaccination with two different types of vaccines and there is a strong possibility that the above concerns can be addressed. For this, we have selected mRNA (Pfizer) and Inactivated Virus (Sinopharm) vaccines. There are several animal studies where immune response with heterologous vaccines was much stronger and lasting as compared to single vaccines but no human study. We strongly believe that with heterologous vaccines there will be a much stronger and longer-lasting immune response and maybe more effective against the variants. Considering the lack of human studies, I had both Sinopharm and Pfizer vaccines and will monitor humoral immune response.

Keywords: Heterologous, Homologous, Cytokine Storm, CD4+ Cells, CD8+ Cells, Pfizer (mRNA), Sinopharm (inactivated Virus Vaccine).

INTRODUCTION

There are 5 different platforms for Covid-19 vaccines and about 16 different vaccines developed. All these vaccines train the immune system to recognize the virus differently and lead to developing more strong and durable immune response.

The platforms and vaccines in each platform are as follows:

1-RNA based vaccine eg Pfizer, Moderna, CureVac G

2-Viral Vector (adenovirus-non-replicating) eg AstraZeneca, CanSino, Sputnik, Janssen

3-Inactivated Virus eg Sinopharm, Sinovac, etc.

4-Protein subunit eg Novavax

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5-DNA based Vaccine eg Zydus Cadila

All the Covid-19 vaccines are widely available and require two doses except Johnson & Johnson's (J&J) which needs a single dose. The first dose primes the immune system, and the second dose is administered usually on the 21st day after the first dose which boosts it.

Homologous prime-boost Vaccination

All the Covid-19 vaccines widely available and usually need 2 doses about 21 days apart. The first dose primes the immune system, and the second dose is given 21-28 days apart, which boosts it. Using the same vaccine preparation for the first and 2nd dose is called homologous prime-boost.

Heterologous Prime-Boost Vaccination or Mixing and Matching Covid-19 Vaccination

What is The Mixing and Matching of Vaccines?

There is an interesting example of mixing-and-matching as wearing office attire on top (coat, matching tie, and waistcoat) and pajama pants on bottom. This is on the lighter note and here are the following types of Immunoboost strategies for the Covid-19 Vaccination schedules.

In some countries, there may be a shortage of vaccines after the first dose of the vaccine, and people can't have the 2nd dose of the same vaccine after 21 days. If the 2nd dose of the same vaccine is given it is called Homologous Vaccination. Public Health Department England after the 2nd dose of the vaccines was delayed for up to 12 weeks suggested that these people can have the 2nd dose of some different platform vaccine to have some protection. Using different vaccine preparations is called heterologous prime-boost. For example, the first dose of AstraZeneca is given, and then after 21 days instead of AstraZeneca Vaccine we give a shot of Pfizer Vaccine, and this is Heterologous Boost Vaccination.

Enhanced Heterologous Immuno-Boost Vaccination (We recommend)

- We recommend that one should get 1st dose of one type of Covid Vaccine and then after 21 days should get the 2nd Dose of the same vaccine (e.g., platform 3).
- Then one should get 2nd Vaccine from a different Covid Vaccine platform e.g., dose 1, and then a dose 21 days after the 1st dose from the platform (e.g., from the platform).

Who is testing the mix-and-match theory?

The Com-Cov study launched in February 2021 with participants receiving alternating doses of the Oxford AstraZeneca and Pfizer vaccines.

Currently, in the UK, an eight-arm study assessing the mix-and-match theory is underway. The ambitious University of Oxford-run trial called **Com-COV2** is testing various combinations of the vaccines currently approved in Britain – Pfizer-BioNTech, AstraZeneca, and Moderna, as well as Novavax's candidate, which is expected to be approved in the coming weeks. The trial will enroll 1.050 adult subjects aged 50 years or older who received their first dose of a Covid-19 vaccine in the past eight to 12 weeks.

Covid-19: Moderna and Novavax vaccines to be tested in mixing vaccines trial Elisabeth Mahase (Mahase, 2021).

Gamaleya and AstraZeneca have registered a pair of clinical trials in which volunteers will receive a dose of AstraZeneca's vaccine and another of Sputnik V.

One trial in Azerbaijan is underway, and a second in Russia is still under review by the country's ministry of health.

Spain to start a clinical trial on mixing COVID Vaccines

Spain's public epidemiological institute announced on Monday that it will begin a clinical trial to study the effects of combining the AstraZeneca and Pfizer COVID-19 vaccines. What happens if you get one jab from AstraZeneca and one the next one from Pfizer? Spain hopes to get data by late May-Alyssa McMurtry (Link 1).

VACCINES and Role of Neutralizing Antibodies

SARS-CoV-2 attaches to host cells by binding the S protein to ACE2 receptors (angiotensin-converting enzyme 2) which are the viral receptors on the host cells (Speiser et al., 2020). These S proteins must be primed by the furin and serine proteases TMPRSS2 & TMPRSS4 present in the host cells then only the spikes attach to the host cells (Hoffmann et al., 2020). Binding takes place by receptor binding proteins (RBD) on a spike so most of the neutralizing antibodies are in infection or vaccines are directed against the receptor-binding domain n the spike which blocks the attachment of the virus to the host cells (Barnes et al., 2020). Most of the COVID-19 vaccines use this strategy of generating antibodies against the RBD component of the spike protein S (Zhang et al., 2020). Vaccines produce both the humoral and cellular immune response eg Pfizer-BioNTech, Moderna & Oxford-AstraZeneca) generate anti-spike antibodies and cellular immune responses specific to SARS-CoV-2 (Zhu et al., 2020).

The individual with higher levels of anti-spike antibodies have higher levels of SARS-CoV-2 specific T cells secreting interferon gamma (Schwarzkopf et al., 2021). Several studies have demonstrated the long-term protective roles of vaccines against COVID-19 (Link 2; Link 3).

COVID-19 Vaccine

Vaccines deliver the immunogen which may be adenoviral vector, inactivated/dead virus, or messenger RNA. These when administered train the immune system to recognize the virus when a person is exposed to COVID-19 as a pathogen and induce immune response as follows (Link 2; Link 4):

CD4+ T helper cells that in turn stimulate B cells.

B-cells stimulated to produce virus-specific neutralizing antibodies.

CD8+ T cytotoxic T cells to recognize and kill the cells infected by the SARS-CoV-2 VIRUS

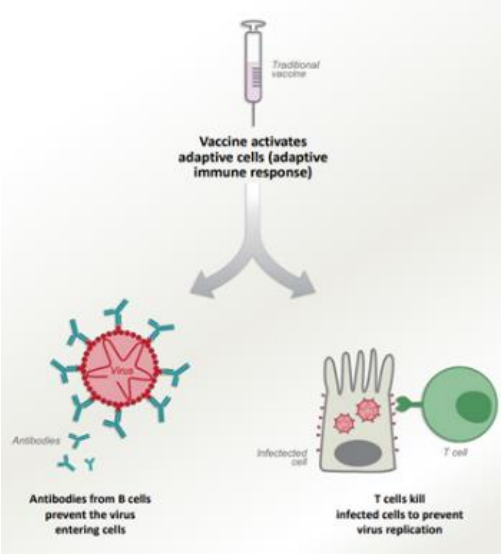

Figure 1. Vaccination (Link 5)

About 140 candidate vaccines are being developed for SARS-CoV-2 in the world following are being used in different countries different types of vaccines (Link 4):

- **Adenoviral Vector vaccines** such as Oxford-AstraZeneca, Gamaleya-Sputnik V, Jhonson & Johnson, CanSinoBIO.
- **Inactivated whole-virus eg Sinopharm-BIBP2, Sinovac. Based on inactivated dead-virus induces strong antibody response but has no or low cellular response** (Davies et al., 2021; Finkelstein et al., 2021).
- **mRNA-based Pfizer-BioNTech, Moderna use mRNA coding for a viral protein leads to rapidly developing strong cellular immunity but the disadvantage is that there is a low antibody response** (Link 6).

All the COVID-19 vaccines induce the adaptive immune response by generating a humoral response with specific anti-spike antibodies by the B cells. These antibodies bind with the virus antigens and stop the virus from attaching to the host cells or opsonize or attach to host cells or even tag to the virus for destruction by macrophages and killer cells. There is also a generation of cellular immune response by the generation of Cytotoxic T cells which destroy the cells infected by the virus. Some B and T cells become Memory B and Memory T cells remain in the body for a longer time and provide immunological memory (Link 2; Link 4).

Covid-19 Vaccination and Natural Infection Differences

Messenger RNA vaccines induced elevated antibody (Ab) reactivity levels against the Receptor Binding Domain (RBD) of SARS-CoV-2 spike and cross-reactive responses against SARS and MERS RBD domains. The observed seroconversion level and breadth induced by the mRNA vaccines is much greater than that induced by natural infection. Differences were noted in the Ab responses induced by the vaccine compared to natural exposure[16]. The nucleocapsid protein is the main antibody produced with natural exposure. The nucleocapsid is not a component of the mRNA vaccines so NCP antibodies are not increased with messenger RNA vaccines, so it is taken as a biomarker for natural exposure in persons having such vaccines (Assis et al., 2021).

Covid-19 Vaccination Strategies

At present homologous prime boosting schedule is being followed by almost all the vaccines except Johnson & Johnson and Singly-Dose Sputnik light Covid Vaccine where only a single dose is required and boosting dose is not required after 21 days while single-dose vaccines. The non-availability of the 2nd dose of the same vaccine has led to the idea of mixing and matching of the Vaccines or Heterologous Prime-Boost Vaccination. Trials in the many UK and many other countries are underway for the efficacy of this strategy of vaccination.

Homologous prime-boost Vaccination

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Heterologous Prime-Boost Vaccination or Mixing and Matching Covid-19 Vaccination

What is The Mixing and Matching of Vaccines?

Using different vaccine preparations is called heterologous prime-boost. For example, the first dose of AstraZeneca Covid dose is given, and then after 21 days instead of AstraZeneca Covid Vaccine we give a shot of Pfizer Vaccine and this is Heterogenous Boost Vaccination.

In some countries, there may be a shortage of vaccines after the first dose of the vaccine, and people cannot have the 2nd dose of the same vaccine after 21 days. Public Health Department England after the 2nd dose of the vaccines was delayed for up to 12 weeks suggested that these people can have a 2nd dose of different platform vaccine to have some protection. This has lead the scientists of different countries to have projects of mixing and matching the different covid vaccines.

Currently, in the UK, an eight-arm study assessing the mix-and-match theory is underway. The ambitious University of Oxford-run trial called Com-COV2 is testing various combinations of the vaccines currently approved in Britain – Pfizer-BioNTech, AstraZeneca, and Moderna, as well as Novavax's candidate, which is expected to be approved in the coming weeks. The trial will enroll 1,050 adult subjects aged 50 years or older who received their first dose of a Covid-19 vaccine in the past eight to 12 weeks (Mahese, 2021; Link, 7).

The six new arms are:

- AstraZeneca vaccine dose followed by AstraZeneca vaccine dose, 4 weeks apart
- AstraZeneca vaccine dose followed by Pfizer vaccine dose, 4 weeks apart
- Pfizer vaccine dose followed by Pfizer vaccine dose, 4 weeks apart
- Pfizer vaccine dose followed by AstraZeneca vaccine dose, 4 weeks apart
- AstraZeneca vaccine dose followed by AstraZeneca vaccine dose, 12 weeks apart
- AstraZeneca vaccine dose followed by Pfizer vaccine dose, 12 weeks apart
- Pfizer vaccine dose followed by Pfizer vaccine dose, 12 weeks apart
- Pfizer vaccine dose followed by AstraZeneca vaccine dose, 12 weeks apart

Gamaleya and AstraZeneca have registered a pair of clinical trials in which volunteers will receive a dose of AstraZeneca's vaccine and another of Sputnik V (Link 8).

There have been various studies about the mixing and matching or Heterologous-prime boosting with very favorable results about the efficacy and safety of this vaccines (Link 8; He et al., 2021; Spencer et al., 2021).

Enhanced Heterologous Immunoboost-Boost (EHIB) Vaccination (We recommend)

We recommend that one should get 1st dose of one type of Covid Vaccine and then after 21 days should get the 2nd Dose from the same vaccine (eg platform 3 – Inactivated virus).

Then one should get 2nd Vaccine from a different Covid Vaccine platform eg dose 1 and then dose 2 after 21 days of the 1st dose from may be platform 1 (RNA-based vaccine).

One cycle of Homologous Prime Boost Vaccine from one platform to be followed by one cycle of Homologous Prime Boost Vaccine from another platform.

Background

The concept is similar to the above-mentioned Heterologous Prime-Boost Vaccination where one dose of one platform of vaccines is administered and then after 21 days or more 2nd dose of vaccine from another platform is administered. But we recommend having first complete homologous vaccination from one platform following the recommended schedule of 1st dose and then 2nd dose of the same platform vaccine after 21 days following the same schedule from the vaccine of another platform.

Some vaccines produce good humoral responses and not good cellular e.g. killed virus COVID-19 Vaccines (Sinopharma) while others produce good cellular immune responses eg mRNA vaccines (Pfizer/Moderna) while not good humoral responses responses (Link 2; Link 4). Both humoral and cellular immune responses are important for protection against COVID-19 infections. T cell responses are last longer and persist even when the protective antibodies are declining or absent (Mahese, 2021; Polack et al., 2020; Schwarzkopf et al., 2021). Both memory Tcell and B cell responses specific to SARS-CoV-2 have been found up to 6 months (Mahese, 2021; Schwarzkopf et al., 2021; Ramasamy et al., 2020; Zuo et al., 2021). Studies have demonstrated the presence of a higher titer of cellular immune responses to SARS-CoV1 and MERS-CoV infection several years after the infection (Bonilla and Oettgen, 2010).

Our Concerns

The Inactivated/Killed SARS-CoV-2 vaccines generally induce only antibody-mediated immunity and not cell-mediated immunity, while messenger RNA and adenovector-based vaccines (Cansino) Vaccines produce stronger T Cellular response and relatively low neutralizing antibody response (Link 3; He et al., 2021; Yang et al., 2021). So having a homologous vaccine one will have a humoral response more or the cellular response more but in fact, for protection both humoral and cellular responses are required. Several studies have demonstrated heterologous prime-boost vaccines give better immune responses than the single or homologous vaccination schedules (Wang et al., 2008; Gil et al., 2013; Excler JL, Kim, 2019). Because Heterologous Prime Boost Vaccination immune responses are better than the homologous immune responses, we strongly recommend the Heterologous Immuno-Boost Vaccination regimen which is expected to have superior and stronger humoral and T cellular immune response.

Antibodies produced by the homologous schedule are mostly neutralizing antibodies and the titer of the antibodies in some people is low after having vaccine jabs as recommended. The antibodies start declining after some time-varying with the type of vaccines e.g. those with 95% efficiency will be expected to maintain the efficacy of 77% by day 250 (8 months) while those with an efficiency of 70% will be predicted to drop efficacy to 33% after day 250 (8 months) (Khoury et al., 2021). The Sinopharm has efficacy of 79.4% (Dyer, 2021), considering above data (Khoury et al., 2021) Sinopharm vaccine will have predicted efficacy of 33.8% after day 250 or 8 months and Pfizer-BioNTech is 95% (Olliaro et al., 2021) will have predictive efficacy of 77% by 8 months after vaccination (Khoury et al., 2021). It is expected that with the Enhanced Heterologous Immuno-Boost Vaccination (EHIB) strategy the combined effect of both Sinopharm and Pfizer will maintain the higher efficacy level for a longer time than 8 months due to stimulating both the humoral and cellular immune system simultaneously and developing the memory B & T Cells.

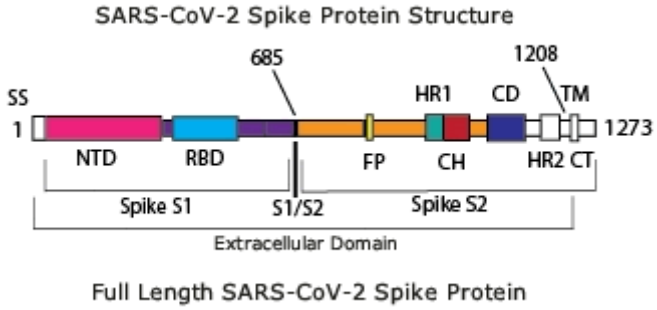

Figure 2. Polyclonal anti-spike antibodies against spike protein (each epitope 7-12 amino acids long and whole spike protein is thousands of aminoacids) (Link 9).

Anti-spike antibodies are produced against several regions (epitopes) on the spike protein and each epitope is about 7-12 amino acids (there are thousands of amino acids on the spike of the SARS-CoV-2 grouped into epitopes). If the SARS-CoV-2 vaccine is specifically against the spike then the individual will generate antibodies to various spike epitopes like NTD, RBD, S1, and S2 (Polyclonal antibodies). Neutralizing antibodies against RBD (Receptor Binding Protein) which prevent attachment of the virus spike to ACE2 receptor (Berry et al., 2010). Both RBD and NTD (N-terminal domain) binding antibodies are important for protection and this response of polyclonal antibodies is more robust, targeted, and exceeds response to infection

(Chi et al., 2020; Finkelstein et al., 2021), that is why vaccination provides superior protection against the disease as compared to the immune response to infection. These two antibodies co dominate as B-Cell targets on the viral spike proteins and both are important for protection against the infection (Pierce et al., 2021). B cells after the infection, vaccination (higher with mRNA vaccination) not only produce the antibodies against the RBD but also produce against the NTD (Amanat et al., 2021).

Considering the above EHIB Vaccination will provide better protection since there will be stronger production of both RBD and NTD neutralizing antibodies required for better protection.

Cross Reactivity

mRNA vaccine polyclonal antibodies have cross-reactive epitopes and are found to be effective against more common seasonal CoVs infections like OC43 (1960) and HKU1 (2004). This phenomenon is **immune imprinting** described in influenza virus immune response and as **a back boost** to Beta-corona viurses (Aydillo et al., 2020; Song et al., 2021). *(Back boost can be explained that suppose one has vaccine today for future infections it will not only prevent the targeted infections but will prevent the older strains too).*

So, taking the mRNA vaccines in addition to killed virus vaccine EHIB Vaccination will not only have increased antibody production and prevent the infection effectively but will also prevent other COVs infections which are quite prevalent and causing flu-like symptoms or we commonly say suffering from the cold due to combined effect of RBD and NTD antibodies.

Along with antibodies more effective Cellular immunity with the generation of memory T Cells with longer life and may even last several years.

EHIB Vaccination may be more effective in the variants of SARS-CoV-2

There can be mutations in small regions of amino acids on RBD (Receptor Binding Domain) and NTD (N Terminal Domain) regions while NTD has higher mutations in the three variants such as VOCs B.1.1.7, B.1.351, and P1 (Davies et al., 2021; Tegally et al., 2020; Amanat et al., 2021).

It is important to note that if the mutation increases the affinity of the virus to bind with the receptor more strongly there is also increased affinity of the antibodies to bind more strongly with the virus. Mutation N501Y in variant B.1.1.7 (very virulent) increases the virus capability to bind more strongly with the ACE2 receptor it is also increasing the affinity of the virus to bind more strongly with the antibodies (Davies et al., 2021; Tegally et al., 2020; Amanat et al., 2021). Mutations in the RBD region eg N501Y mutation found in B.1.1.7 variant have 5 fold affinity to ACE2 receptors and increase its infectivity. While variant having mutation E484 has 4-fold reduced affinity for the ACE2 receptors, so it explains that variants having this mutation rarely causes spread of the disease and is lesser virulent. On the other hand, variant B.1.1.7 having mutation E484K (UK Variant) it will be lesser virulent and more easily prevented from attachment by the anti-RBD antibodies. It was lesser virulent and there was lesser severity of symptoms in the patients. So, the mutations which increase the affinity of the RBD region increase the infectivity of the virus (Choi et al., 2020; Amanat et al., 2021).

Explanation: RBD antibodies are effective against the mutations too, suppose one has developed anti-RBD antibodies on the spike if 4 out of 6 different epitope anti-spike antibodies are not functioning due to mutations there may be antibodies to 2 epitopes on the spike which may be effective and prevent attachment of the virus to the ACE2 receptor. Most RBD antibodies may not effective but some antibodies prevent the attachment since mutation has taken place in only a small part of the virus (a few amino acids in RBD and not in all of the RBD regions on the spike, whereas there are thousands of the amino acids are there on the spike.) So although there is a mutation of one or two amino acids in the RBD region while the functional part of the RBD of the virus is still the same so one or two types of antibodies may not be effective against the mutated part of the RBD but there are several other antibodies to the several epitopes on this region and those can effectively attach to the RBD region and prevent attachment to the ACE2 receptors on the host cells. Thus, increased number and variety of RBD antibodies to spikes in the killed virus vaccine (Sinopharm due to different configuration during attenuation or killing of the virus) and mRNA (Pfizer) vaccines (EHI-Boost Vaccines can be more effective in the variants as compared to the homologous vaccines.

The author recommended Enhanced Heterologous Immuno-Boost Vaccination strategy for COVID-19 and believed it to have a superior immune response as compared to homologous prime-boost (currently being followed) and Heterologous Prime-boost strategies. Some apprehensions adopting such a schedule may be dangerous and may cause Antibody-Dependent Enhancement and cytokine storm and was strongly by scientists and physicians. The author told his family and colleagues in the USA and UK that he is going to try the combination and the idea was strongly opposed. But being an immunologist and allergist, he strongly believed that combination could generate a superior humoral and cellular immune response and will not cause Cytokine storm or Antibody-Dependent Enhancement. He was COVID-19 negative by PCR and planned vaccination to prove his thesis along with the post-vaccination anti-spike antibody tests.

COVID-19 Vaccination in Immunocompromised patients

Several studies have shown the effectiveness of the vaccines against COVID-19, But these studies did not include the immunocompromised patients. One such study (CoVICS) is launched by UPMC (Link 10) and another study (OCTAVE trial) (Link 11) has been launched at the University of Birmingham. We strongly believe that the Enhanced Heterologous Immuno-

Boost (EHIB) strategy of COVID-19 vaccination can be more effective in immunocompromised patients if administered along with a high dose of Vitamin C. This dual combination of vaccines is going to open/pave More And better Possibility of infection prevention. Although randomized large number blinded studies may need to be done there is a signal and a sign of hope. We recommend further studies to prove the effectiveness of the EHIB strategy in immunocompromised patients.

Record of COVID-19 Enhanced Heterologous Immuno-Boost (EHIB) Vaccination along with antibodies tests)

(Since no study has been done till now so author volunteered for this study, had EHIBV and post-vaccination antibodies tests with his serum.)

- Initially, the author was COVID-19 negative as per the PCR test.
- Anti-spike antibodies test (post-vaccination antibodies Test) was done on 24/02/2021 and was NEGATIVE (<0.40U/mL).
- Sinopharm Vaccination 1st dose on 6/3/2021.
- Sinopharm Vaccination 2nd Dose on 27/03/2021.
- Anti-spike antibodies test (post-vaccination antibodies Test) done on 27/03/2021 and titer was 3.93U/ml (only mild increase in 21 days after the 1st dose of Sinopharm).
- Pfizer Vaccine 1st dose on 24/3/2021.
- Pfizer's 2nd dose on 14/04/2021 which is exactly 21 days after the first dose of the Pfizer Vaccine.
- Anti-spike antibodies test (post-vaccination antibodies Test) done on 10/04/2021 and titer was 682.00U/mL, 14 days after 2nd dose of Sinopharm vaccine and 17 days after 1st dose of Pfizer Vaccine.
- Anti-spike antibodies test (post-vaccination antibodies Test) done on 27/04/2021 and titer was >2500.00U/mL, 27 days after 2nd dose of Sinopharm vaccine and 13 days after 2nd dose of Pfizer Vaccine.
- Anti-Spike antibodies test 57 days after 2nd dose of Sinopharm vaccine and 43 days after 2nd dose of Pfizer Vaccine. Antibodies were detected with 1:3 dilution of the serum titer 1588U/mL (detection limit was 2500U/mL) and after multiplication, with factor, it was 4764U/mL.

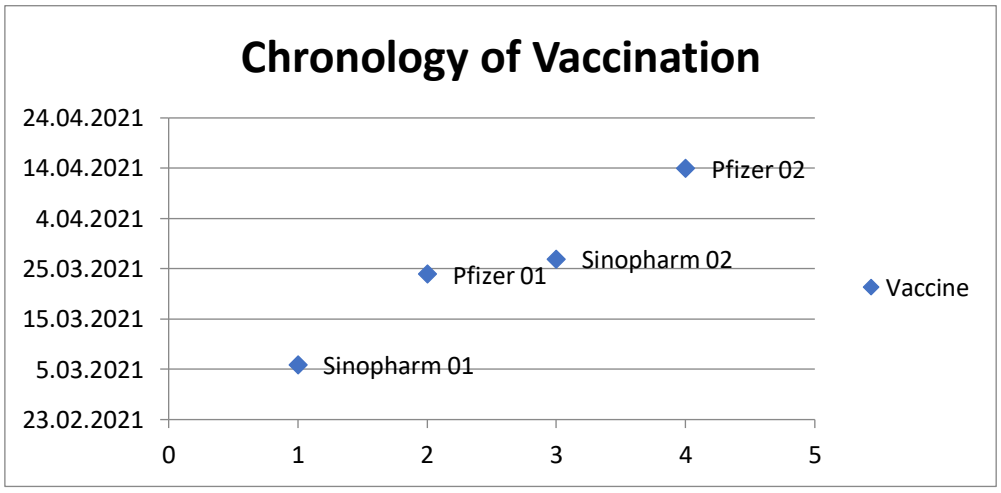

Figure 3. Chronology of Vaccination

0-pcr test was negative for SARS-CoV-2 and no IgG antibodies were detected against COVID-19 on 2/24/2021.

1-1st dose of Sinopharm (01) administered on 3/6/2021

2-1st dose of Pfizer (01) administered on 3/24/2021

3- 2nd dose of Sinopharm (02) administered on 3/27/2021

4-2nd dose of Pfizer (02) administered on 4/14/2021

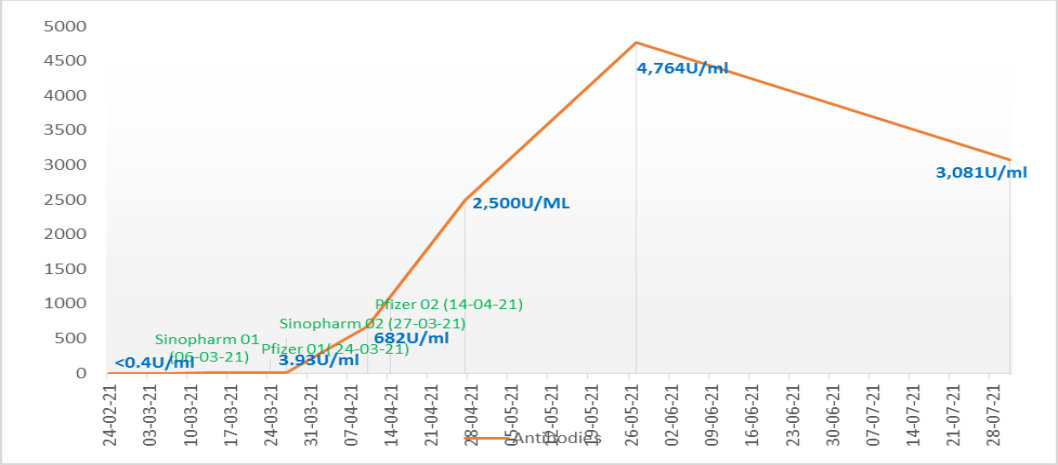

Figure 3. Antibodies

- *Initially, COVID-19 negative as per the PCR test.*
- *Anti-spike antibodies test (post-vaccination antibodies Test) was done on 24/02/2021 and was NEGATIVE (<0.40U/mL).*
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- *Anti-spike antibodies test (post-vaccination antibodies Test) done on 27/04/2021 and titer was >2500.00U/mL, 27 days after 2nd dose of Sinopharm vaccine and 13 days after 2nd dose of Pfizer Vaccine.*
- *Anti-spike antibodies test (post-vaccination antibodies Test) done on 27/05/2021 and titer was >2500.00U/mL, 57 days after 2nd dose of Sinopharm vaccine and 43 days after 2nd dose of Pfizer Vaccine. Antibodies detected with 1:3 dilution of the serum titer 1588U/mL (detection limit was 2500U/mL) and after multiplication, with factor it was **4764U/mL***

Explanation (Figure 3, Table 1)

- Anti-spike antibodies and PCR test for COVID-19 were negative before the vaccination and there was little increase in these antibodies (3.93U/mL) on 27th March ie 21 days after the 1st dose of Sinopharm vaccine (date of 2nd dose of the same vaccine).
- There was a moderate increase of 682.00U/mL of these antibodies on 10/4/2021 which is 14 days after the 2nd dose of Sinopharm and 17 days of the 1st dose of Pfizer.
- There was a much higher increase to >2500.00U/mL on 27/04/2021 which is 27 days after the 2nd dose of Sinopharm and 13 days after the 2nd dose of Pfizer.

- Since the detection limit of the Elecys (Anti-SARS-CoV-2 S) Immunoassay done on Cobas e 411 instruments using electrochemiluminescence Immunoassay (ECLIA) is 25000.00U/mL and the previous test on 27/04/2021 titer was >2500U/mL which was not exact titer and could not be interpreted. We decided to make a 1:3 dilution with serum and after mixing well was sent to the same lab for the tests.
- Anti-spike antibodies test (post-vaccination antibodies Test) done on 27/05/2021, 57 days after 2nd dose of Sinopharm vaccine and 43 days after 2nd dose of Pfizer Vaccine. Antibodies were detected with 1:3 dilution of the serum titer 1588U/ml (detection limit was 2500U/mL) and after multiplication, with factor, it was 4764U/mL.

Antibody Response against Vaccination (Figure 3, Table 1)

The data shows that 1st dose of Sinopharm generated an insignificant titer of anti-spike antibodies (3.93U/mL) till 21 days after the first dose of Sinopharm Vaccine while 14 days (2 weeks) after 2nd dose of Sinopharm vaccine there was a moderate increase in anti-spike antibodies (titer 682.U/mL) indicating there is an only moderate increase in antibodies two weeks after the 2nd dose of Sinopharm or 17 days after 1st dose of Pfizer. This could have been the combined effect of the 2 doses of Sinopharm Vaccine and one dose of Pfizer. It could be immunopotentiation of Sinopharm (two doses) and a single dose of Pfizer Vaccine otherwise the titer of Sinopharm Vaccine could be lesser.

It is important to note that 13 days (2 weeks) after the 2nd dose of Pfizer and 27 days (4 weeks) after the 2nd dose of Sinopharm, much higher levels of anti-spike antibodies were detected >2500.00U/mL. Although killed virus vaccines are normally known to generate much higher levels of anti-spike antibodies while mRNA vaccines generate a much lower level of antibodies ad higher levels of cellular immune response81,85. But here we have noticed that the killed virus vaccine (Sinopharm alone does not generate a higher level of antibodies even after two jabs of the vaccine and 2nd dose of mRNA after both doses of Sinopharm potentiated the immune response and much higher levels of antibodies were generated.

Follow up of the Anti-Spike antibodies test level on 27/05/2021, 57 days after 2nd dose of Sinopharm vaccine and 43 days after 2nd dose of Pfizer Vaccine. Antibodies were detected with 1:3 dilution of the serum titer 1588U/mL (detection limit was 2500U/mL) and after multiplication, with factor, it was 4764U/mL. The exact titer on 27/04/2021 could have been different but due to constraints of the kit with an upper detection limit of 2500U/mL it could not be confirmed. But this titer with 1:3 dilution will be followed up every 2-3 monthly. This will help to determine that the EHIB Vaccination schedule has:

- Immune boosting potential
- Kinetics of the anti-spike antibodies with this strategy.
- How long the acceptable protective antibodies persist in the body after the EHIB (Enhanced Heterologous Immuno-Boost Vaccination against COVID-19.
- Compare with other vaccination schedules like Homologous Prime Boost and Heterologous Prime Boost strategies

Table 1. Record of the EHIB Vaccination and antibodies

DATE	**LAB**	**MRN NO**	**RESULTS**
24/2/21	Shaukat Khanum	001-80003395742 *BEFOR VACCINATION*	Coronavirus Antibody Test-Post Vaccination (SARS-CoV-2S) <0.40U/mL Interpretation: -Non Reactive
6/3/2021	Sinopharm FIRST DOSE vaccine given	CODE 2620 in PIMS	No side effects
18/3/21	REHMAN	2118375322	PCR SARS-CO-V2 NEGATIVE
24/3/21	MEDICLINIC DUBAI	20454398	219 Novel Corona Virus-(sars-Cov-2) Not detected

Table 1 continued

DATE	LAB	MRN NO	RESULTS
24/3/2021	1st DOSE Pfizer Vaccine given	MRN # 921025679	No side effects
27/3/2021	2nd DOSE Sinopharma vaccine given	Code:-5975	No side effects
27/3/2021	Post-vaccination SARS-COV-2S ANTIBODIES BY Elecys(Anti-SARS-COV-2 S-Quantitative	MRN 001-80003395742	Titer 3.39U/mL (Day 21 after 1st dose of Sinopharm) *On the same day of having 2nd dose of SINOPHARM & 3 days after Pfizer Vaccine*
10/4/2021	Post-vaccination SARS-COV-2S ANTIBODIES BY Elecys(Anti-SARS-COV-2 S-Quantitative	MRN 001-80003395742	-Titer (Day34) *14 Days after 2nd Dose of Sinopharm and 17 days after Pfizer 1st Dose*
14/4/2021	2nd dose Pfizer	MRN # 921025679	Pains and aches, feverish feeling, and drowsiness lasted for about 6 hours on day one only
27/4/2021	Post-vaccination SARS-COV-2S ANTIBODIES		-Titer 2500.00 U/mL (Day 51) *27 Days after 2nd Dose of Sinopharm and 13 days after Pfizer 2nd Dose*
27/05/2021	Post-vaccination SARS-COV-2S ANTIBODIES BY Elecys(Anti-SARS-COV-2 S-Quantitative-Shaukat Khanum		Titer 4764 (Day 81) *57 days after 2nd dose of Sinopharm vaccine and 43 days after 2nd dose of Pfizer Vaccine. Antibodies were detected with 1:3 dilution of the serum titer 1588U/mL (detection limit was 2500U/mL) and after multiplication, with factor, it was* ***4764U/mL.***
28/7/2021			Titer 3080 U/mL (Day 112)
30/8/2021			Titer 2459.0/mL (Day 144)

CONCLUSION

Antispike antibody response as demonstrated in (Figure 3, Table 1) confirms our thesis that Enhanced Heterologous Immuno-Boost Vaccination can potentiate the immune response and there were enhanced Anti-Spike antibodies. On this basis, there are strong chances that the combined effect will lead to a much superior cellular response and EHIB will be more effective against the variants(as explained that there are numerous epitopes on the RBD region and when enhanced antibodies are generated against the different epitopes of the RBD then there is the possibility that some types of antibodies may not be effective in preventing attachment of the virus to the ACE2 receptors but still there are numerous antibodies generated in response to the EHIB Vaccine strategy that there are sufficient antibodies still present to prevent the variant. (It is to be noted that variants have only a limited number of amino acids variation in epitope while the major region of the RBD is still the same). The author believes that the Enhanced Heterologous Immuno-Boost strategy can generate a much superior and lasting humoral and cellular immune response and will be a basis of further research and implement the EHIB boost Strategy of Vaccination against COVID-

19. But due to financial constraints, cellular studies could not be done in our clinic and this study will lead to further comprehensive studies to prove the thesis.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

Amanat, F., Thapa, M., Lei, T., Ahmed, S. M. S., Adelsberg, D. C., Carreno, J. M., et al., (2021). The plasmablast response to SARS-CoV-2 mRNA vaccination is dominated by non-neutralizing antibodies and targets both the NTD and the RBD. medRxiv. doi: 10.1101/2021.03.07.21253098

Assis, R., Jain, A., Nakajima, R., Jasinskas, A., Kahn, S., Palma, A., et al., (2021). Substantial differences in SARS-CoV-2 antibody responses elicited by natural infection and mRNA vaccination. BioRxiv, 6(1), 132.

Aydillo, T., Rombauts, A., Stadlbauer, D., Aslam, S., Abelenda-Alonso, G., Escalera, A., et al., (2020). Antibody immunological imprinting on COVID-19 patients. MedRxiv. doi: https://doi.org/10.1101/2020.10.14.20212662

Barnes, C. O., West Jr, A. P., Huey-Tubman, K. E., Hoffmann, M. A., Sharaf, N. G., Hoffman, P. R., et al., (2020). Structures of human antibodies bound to SARS-CoV-2 spike reveal common epitopes and recurrent features of antibodies. Cell, 182(4), 828-842.

Berry, J. D., Hay, K., Rini, J. M., Yu, M., Wang, L., Plummer, F. A., et al., (2010, January). Neutralizing epitopes of the SARS-CoV S-protein cluster independent of repertoire, antigen structure or mAb technology. In MAbs (Vol. 2, No. 1, pp. 53-66). Taylor & Francis.

Bonilla, F. A., Oettgen, H. C. (2010). Adaptive immunity. Journal of Allergy and Clinical Immunology, 125(2), S33-S40.

Chi, X., Yan, R., Zhang, J., Zhang, G., Zhang, Y., Hao, M., et al., (2020). A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. Science, 369(6504), 650-655.

Choi, B., Choudhary, M. C., Regan, J., Sparks, J. A., Padera, R. F., Qiu, X., et al., (2020). Persistence and evolution of SARS-CoV-2 in an immunocompromised host. New England Journal of Medicine, 383(23), 2291-2293.

Davies, N. G., Abbott, S., Barnard, R. C., Jarvis, C. I., Kucharski, A. J., Munday, J. D., et al., (2021). Estimated transmissibility and impact of SARS-CoV-2 lineage B. 1.1. 7 in England. Science, 372(6538), eabg3055.

Dyer, O. (2021). Covid-19: Chinese vaccines may need changes to improve efficacy, admits official. British Medical Journal, 373, n969.

Excler, J.L., Kim, J.H. (2019). Novel prime-boost vaccine strategies against HIV-1. Expert Rev Vaccines, 18, 765–779.

Finkelstein, M. T., Mermelstein, A. G., Parker Miller, E., Seth, P. C., Stancofski, E. S. D., Fera, D. (2021). Structural analysis of neutralizing epitopes of the SARS-CoV-2 spike to guide therapy and vaccine design strategies. Viruses, 13(1), 134.

Gil, A., Shen, S., Coley, S., Gibson, L., Diamond, D. J., Wang, S., Lu, S. (2013). DNA vaccine prime followed by boost with live attenuated virus significantly improves antigen-specific T cell responses against human cytomegalovirus. Human vaccines & immunotherapeutics, 9(10), 2120-2132.

He, Q., Mao, Q., An, C., Zhang, J., Gao, F., Bian, L., et al., (2021). Heterologous prime-boost: breaking the protective immune response bottleneck of COVID-19 vaccine candidates. Emerging microbes & infections, 10(1), 629-637.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al., (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. cell, 181(2), 271-280.

Khoury, D. S., Cromer, D., Reynaldi, A., Schlub, T. E., Wheatley, A. K., Juno, J. A., et al., (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nature medicine, 27(7), 1205-1211.

Link 1. https://www.aa.com.tr/en/europe/spain-to-start-clinical-trial-on-mixing-covid-vaccines/2213975 Accessed 15. 12. 2022

Link 2. COVID-19 vaccine: the eight technologies being tested, https://www.intvetvaccnet.co.uk/blog/covid-19/vaccine-eight-types-being-tested Accessed 15. 12. 2022 Accessed 15. 12. 2022

Link 3. COVID-19 Immunity & Clinical Manifestations CORONAVIRUS (COVID-19) UPDATE NO. 24 https://www.who.int/docs/default-source/coronaviruse/risk-comms-updates/update-24-immuniy-n-clinical-manifestations.pdf?sfvrsn=7c84a6bf_4 Accessed 15. 12. 2022

Link 4. Update on COVID-19 Vaccines and immune response-The latest on the COVID-19 Global situatation and vaccines (WHO) LAST UPDATE: https://www.who.int/docs/default-source/coronaviruse/risk-comms-updates/update73_covid-19-vaccines-and-immune-response.pdf?sfvrsn=7902cc35_5 Accessed 15. 12. 2022

Link 5. https://www.who.int/docs/default-source/coronaviruse/risk-comms-updates/update52_vaccines.pdf?sfvrsn=b11be994_4 Accessed 15. 12. 2022

Link 6. Covid-19 Immunity & Clinical Manifestations- https://www.who.int/docs/default-source/coronaviruse/risk-comms-updates/update-24-immuniy-n-clinical-manifestations.pdf?sfvrsn=7c84a6bf_4 Accessed 15. 12. 2022

Link 7. https://comcovstudy.org.uk/about-com-cov2 Accessed 15. 12. 2022

Link 8. Why mixing vaccines could help boost immunity. New trials on mixing different types of vaccines are underway. Could vaccine combinations help stop variants from bypassing our immune systems? https://www.technologyreview.com/2021/05/06/1024640/why-mixing-vaccines-could-help-boost-immunity/ Accessed 15. 12. 2022

Link 9. https://www.biocat.com/corona-antibodies Accessed 15. 12. 2022

Link 10. https://www.upmc.com/coronavirus/covid-vaccine Accessed 15. 12. 2022

Link 11. https://clinicaltrials.gov/ct2/show/NCT04805216 Accessed 15. 12. 2022

Mahase, E. (2021). Covid-19: Moderna and Novavax vaccines to be tested in mixing vaccines trial. British Medical Journal, 373, n971

Olliaro, P., Torreele, E., Vaillant, M. (2021). COVID-19 vaccine efficacy and effectiveness—the elephant (not) in the room. The Lancet Microbe, 2(7), e279-e280.

Pierce, C. A., Sy, S., Galen, B., Goldstein, D. Y., Orner, E., Keller, M. J., et al., (2021). Natural mucosal barriers and COVID-19 in children. JCI insight, 6(9), e148694

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., et al., (2020). Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. New England journal of medicine.

Ramasamy, M. N., Minassian, A. M., Ewer, K. J., Flaxman, A. L., Folegatti, P. M., Owens, D. R., et al., (2020). 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. The Lancet, 396(10267), 1979-1993.

Schwarzkopf, S., Krawczyk, A., Knop, D., Klump, H., Heinold, A., Heinemann, F. M., et al., (2021). Cellular immunity in COVID-19 convalescents with PCR-confirmed infection but with undetectable SARS-CoV-2–specific IgG. Emerging infectious diseases, 27(1), 122.

Song, G., He, W. T., Callaghan, S., Anzanello, F., Huang, D., Ricketts, J., et al., (2021). Cross-reactive serum and memory B-cell responses to spike protein in SARS-CoV-2 and endemic coronavirus infection. Nature communications, 12(1), 1-10.

Speiser, D. E., Bachmann, M. F. (2020). COVID-19: Mechanisms of vaccination and immunity. Vaccines, 8(3), 404.

Spencer, A. J., McKay, P. F., Belij-Rammerstorfer, S., Ulaszewska, M., Bissett, C. D., Hu, K., et al., (2021). Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. Nature communications, 12(1), 1-8.

Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., et al., (2020). Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. MedRxiv.

Wang, S., Parker, C., Taaffe, J., Solórzano, A., García-Sastre, A., Lu, S. (2008). Heterologous HA DNA vaccine prime—inactivated influenza vaccine boost is more effective than using DNA or inactivated vaccine alone in eliciting antibody responses against H1 or H3 serotype influenza viruses. Vaccine, 26(29-30), 3626-3633.

Yang, S., Li, Y., Dai, L., Wang, J., He, P., Li, C., et al., (2021). Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials. The Lancet Infectious Diseases, 21(8), 1107-1119.

Zhang, J., Zeng, H., Gu, J., Li, H., Zheng, L., Zou, Q. (2020). Progress and prospects on vaccine development against SARS-CoV-2. Vaccines, 8(2), 153.

Zhu, F. C., Guan, X. H., Li, Y. H., Huang, J. Y., Jiang, T., Hou, L. H., et al., (2020). 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, placebo-controlled, phase 2 trial. The Lancet, 396(10249), 479-488.

Zuo, J., Dowell, A. C., Pearce, H., Verma, K., Long, H. M., Begum, J., et al., (2021). Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nature immunology, 22(5), 620-626.