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Review

There is nothing exempt from the peril of mutation – The Omicron spike

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ABSTRACT

The 2019 corona virus disease (COVID-19) has caused a global chaos, where a novel Omicron variant has challenged the healthcare system, followed by which it has been referred to as a variant of concern (VOC) by the World Health Organization (WHO), owing to its alarming transmission and infectivity rate. The large number of mutations in the receptor binding domain (RBD) of the spike protein is responsible for strengthening of the spike-angiotensin-converting enzyme 2 (ACE2) interaction, thereby explaining the elevated threat. This is supplemented by enhanced resistance of the variant towards pre-existing antibodies approved for the COVID-19 therapy. The manuscript brings into light failure of existing therapies to provide the desired effect, however simultaneously discussing the novel possibilities on the verge of establishing suitable treatment portfolio. The authors entail the risks associated with omicron resistance against antibodies and vaccine ineffectiveness on one side, and novel approaches and targets – kinase inhibitors, viral protease inhibitors, phytoconstituents, entry pathways – on the other. The manuscript aims to provide a holistic picture about the Omicron variant, by providing comprehensive discussions related to multiple aspects of the mutated spike variant, which might aid the global researchers and healthcare experts in finding an optimised solution to this pandemic.

1. Introduction

The healthcare paradigm and economic status, across the world is gravely affected by the Corona Virus Disease 2019 (COVID-19), mediated by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus [1]. The overall infections have reached 270 million alongside 5.3 million mortalities, as of 13th December 2021, as per the JHU CSSE COVID-19 Data [2]. Multiple vaccines have been developed and administered to curb the infection spread [3], but the mutations in the viral sequence have brought into the light number of viral variants, which have challenged these efforts by the global healthcare system [4–7]. Multiple strains have been referred to as "variant of concern" (VOCs), among prime variants, by the World Health Organization (WHO). A novel variant (B.1.1.529) was identified and named as Omicron, and designated as a VOC, on 26th November, 2021, by the WHO [8]. The novel Omicron variant was initially identified in Botswana, where by it rapidly spread across the neighbouring nations. Currently, about 26 countries are infected with the Omicron variant [9]. This shows the alarmingly rapid transmission rate of this new variant. The large number of alterations in the Omicron S-protein are significantly responsible for its high transmission and infectivity [9]. Numerous mutations have been accumulated in the Omicron variant, specifically in the spike protein, which initiates the host cell entry, to mediate infection. The receptor binding domain (RBD) of the spike protein of the virus comprise of an overall of 30 mutations [8,10,11].

The manuscript details the story of the mutated spike, aiming to provide a wide compendium of data, associated with the novel Omicron variant. The text begins with the binding of the RBD domain of the

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variant to ACE2, which entails the use of ACE2 orthologues and explores the reason, as to why the transmission rate of this variant exceeds to such a great extent, as compared to the original strain. The effect of the variant on the pre-existing antibodies is another significant aspect discussed in the manuscript, followed by a discussion of "host jumping" of the variant. The objective of the authors is not only to investigate the details of the Omicron variant, but also validate the potential of existing vaccines and booster doses in mitigating the spread of Omicron variant. The interesting facet is formed by a comprehensive data on the therapeutic targets and candidates, expected to offer desired protection against the Omicron variant, primarily discussing the kinase inhibitors, paxlovid, TMPRSS2 – cathepsin entry pathways and their comparative evaluation, as well as medicinal phytocompounds. Therefore, all in all the authors aim to provide a holistic picture of the Novel Omicron variant, to the global researchers, and aid their efforts to find an optimized escape to the problem, before it devours the entire world into its depths.

2. The story of the mutated spike

Between November 22nd and 23rd of 2021, the initial sequences of the Omicron variant were incorporated into the Global Initiative on Sharing All Influenza Data database, which was derived from Batswana and South African patients [12]. Later, there was a rapid escalation in the number of deposited sequences of Omicron, alongside the detection of virus in Asia, USA and Europe, due to infected air travelers. Evaluation of the genomic sequence of the Omicron variant portrayed significant differences from other known variants of the SARS-CoV-2, significantly in isolated population of humans, unidentified species of animals and immunocompromised patients [13]. Unlike the Wuhan-Hu-1 spike, the Omicron spike, was reported to exhibit 37 mutations, wherein, 13 alterations are characterized to be unique [14]. To be specific, the NTD is reported to comprise of 11 mutations, consisting of 1 insertion and 6 deletions. The mutations ins214EPE as well as N211Δ are considered to be unique [12]. The binding potential to the antibody might be reduced or the spike expression might be elevated by the deletions, which are found in other VOC [15–19]. Moreover, the RBD has been considered to incorporate 15 mutations [20], out of which S371L, S375F, G339D and S373P, are unique, while others were identified to regulate binding to ACE2, or also antibody invasion [15–19]. Five unique alterations are located in the S2 subunit, whereas five mutations are also contained between S1/S2 and RBD, including the unique P681H and T547K mutations that might regulate the chasm at S1/S2 location by protease Furin of the host [21,22]. Overall, the S2 subunit comprises of five exclusive mutations [14].

2.1. ACE2 binding and entry into cell lines

The vesicular stomatitis virus (VSV) entities, pseudo-typed with 127 S-proteins of the SARS-CoV-2, were used for evaluation of host cell entry, where these pseudo-typed entities imitate the primary characteristics of the viral entry into the cells targeted, comprising of choice of receptor and protease as well as antibody-mediated neutralization [23,24]. The questions raised was if the Omicron spike exhibited any difference from the VOC spike, in terms of effectiveness of entry as well as choice of target cell [12]. The evaluation of S-protein from viral B.1 was carried out in parallel, due to early circulation of the virus in the pandemic ad absence of mutations reported in the S proteins of VOCs. Multiple cell lines were used for the evaluation of cell tropism, such as 293 T, Huh-7, Calu-2, Vero, A547 and Caco-2, which were greatly vulnerable to entry, promoted by VSV-G as well as SARS-CoV-2 B.1 spike [12]. Furthermore, the VOC protein-mediated cell entry was evaluated, but only minor differences were observed. Therefore, the delta spike promoted elevated entry into Caco-2 and Calu-3 cells [25], whereas the spike of the Omicron variant facilitates entry into 293 T, Huh-7 and Vero cells. Moreover, the comparative access into the A549-ACE2 cells, mediated by Omicron spike, was more efficient than the entry facilitated by other S protein [12].

2.1.1. Cell entry characterized by the use of orthologues of ACE2 from varying species of animals

Another question that arises is, if the spike of the Omicron variant can employ orthologues of ACE2 from varying species of animals, such as masked palm civet, pangolin, horseshoe bats and raccoon dogs, to mediate target cell entry [12]. The B.1 and Delta spike-mediated entry is considered as controls. The VSV-G – mediated entry was not regulated by the expression of ACE2 orthologues, however, in maximum scenarios the delta, B.1 and Omicron spikes were reported to mediate robust cell entry [12]. Furthermore, two exceptions were observed, i.e., a more efficient use of murine ACE2 by Delta spike, unlike the B.1 spike, as well as cell entry mediated by the S-protein of the Omicron with highest potential [12]. Overall, the ACE2 was not able to be used by B.1 spike, from Pearson's horseshoe bat for entry into the cell, whereas the delta as well as, specifically Omicron, employed ACE2 with great efficacy. Moreover, the information reported a wide employment of orthologues of ACE2 by the S-protein of the Omicron for entry into the host cell, that might provide an impression towards great zoonotic capability [12].

2.1.2. What strengthens the RBD-ACE2 binding? RBD mutations?

Greater mutations reported in the COVID-19-causing SARS-CoV-2 spike, have generated critical questions related to the novel Omicron variant. A quantitative evaluation of the stability of the complex formed between RBD and ACE2 was carried out by using computational modelling and simulations, which were then comparatively analysed to that of wild type systems [11]. The multiple quantities, like Vander-Waals contacts, hydrogen bonds, binding free energies, and buried surface areas were used to evaluate the interactions. The mutations at the interface of RBD and ACE2 promote and elevate the tight binding via enhancing the hydrogen bond interaction, as well as increasing buried solvent accessible surface area [11]. The binding interactions between RBD and ACE2 were reported to be slightly stronger in Omicron, in comparison with the wild type system, as shown by the results from the dynamic simulation, as well as quantitative comparative analysis [1]. This data further provides molecular insights to define the basis of elevated infectivity of Omicron variant, based upon its greater affinity towards ACE2 receptor [11]. Furthermore, the effect of neutralising antibodies was reported to be studied on the basis of their interaction to the RBD epitopes, or their ability to compete with ACE2 interactions [26]. However, about 85% of the previously known neutralising antibodies have been reported to be deprived of their potential against the novel Omicron variant [27]. Thus, evaluating the interactions between RBD and ACE2 is important, not only for understanding the mechanism or basis of Omicron infection, but also for predicting as well as designing a suitable therapeutic antibody, which would be effective against the variant. This will further expedite the development of a novel generation of therapeutic antibodies, which would exhibit the potential to fight the immune-escaping mutants [11].

2.2. Antibody resistance

Further, another question comes to mind that, if the S-protein of Omicron can be hindered by the soluble form of ACE2, that upon RBD interaction, inhibits host cell entry, as well as is presently being created for the therapy of COVID-19 [28]. The cell entry, mediated by VSV-G, is not regulated by soluble ACE2, and Omicron spike, delta and B.1 – mediated entry is blocked by it, which depicts the possibility of soluble ACE2 to be used as an optimum therapy for patients infected with the novel Omicron variant [12]. The SARS-CoV-2 infection – blocking multiple recombinants, neutralizing monoclonal antibodies have been identified, where Etesevimab, Casirivimab, Bamlanivimab and Imdevimab, are presently used for COVID-19 treatment [12]. Additionally, Sotrovimab was also reported to block SARS-CoV-2 as well as associated

viruses, and was reported to provide protection against COVID-19 infection. An investigation was carried out to find out if the antibodies, currently, possessed the potential of Omicron S-protein neutralization, on account of multiple alterations incorporated by the Omicron spike, which are identified by these antibodies [12]. The entry promoted by B.1 spike was hindered by all antibodies, in a concentration dependent manner, whereas a control immunoglobulin (Ig) was inactive. On the contrary, the cell entry promoted by the S-protein of Omicron was completely resistant to Imdevimab, Etesevimab as well as Bamlanivimab, while exhibiting extreme resistance against Casirivimab [12]. In accordance with these outcomes, a blend of Etesevimab and Bamlanivimab was unable to block the Omicron spike – mediated host cell entry, whereas the blockage by the mixture of Imdevimab and Casirivimab was ineffective [12]. Contrastingly, Sotrovimab was active against the Omicron variant, even though the blockage was slightly less effective, unlike the B.1 spike. Therefore, the spike of Omicron evidently displays greater resistance to multiple antibodies, employed for the COVID-19 therapy [12]. A study, in its investigation of the impact of monoclonal antibodies against omicron spike protein, revealed failure of activity of 17 out of 19 antibodies tested, even the ones those were approved for use in infected patients [29]. Also, four new spike mutations were recognized, i.e., Q493R, N440K, S371L and G446S, which exhibit significant antibiotic resistance on the variant [29].

2.2.1. Impact on pre-existing antibodies

Studies have been conducted to investigate the impact of alterations in the Omicron S-protein on antibody binding, which are previously formed by infections or immunizations. An in-house R-script was used to find out the Protein Data Bank (PDB) for the structures of the spike protein, which would affect the antibody interaction [11]. Overall, 194 S-protein structures or S-RBD structures have been deposited, in combination with nanobodies/antibodies, within the PDB. The X-ray crystallography have been able to determine 81 structures out of these, while cryo-electron microscopy determined the remaining [11]. Since the IGHV3–53 antibody crystal structure was solved at a great resolution, it was selected, alongside S-RBD with SARS-CoV-2 [30], in order to evaluate the significance of the variant S-RBD alterations on the interaction of antibodies. The criteria behind the selection is dependent on a fact, i.e., upon superimposition of Cα atoms of S-RBD onto the Cα atoms of BNT162b1 (Pfizer vaccine candidate), in complex with ACE2 receptor [31], leads to the root-mean-square-deviation (RMSD) of 12.6 Å. The evaluation depicted that the locations of eight residues, with respect to the variant-specific alterations, are at the interface of S-RBD and ACE2 receptor [11]. The S-RBD/IGHV3–53 interface comprises of the identical 8 residues. Steric interference can be created by the alterations, like Q493R, G496S and G446S, for antibody binding to the S-RBD, while alterations, like Y505H and E484A, might cause deficit of antibody binding. It seems that the overall outcome of the alterations is the alleviated interaction between S-RBD as well as the corresponding antibodies, which indicates that the immunization, that pre-existed, may not safeguard against the Omicron [11]. Furthermore, the cryo-EM arrangement of a neutralizing antibody, directed towards NTD, in combination with prefusion S-glycoprotein of the SARS-CoV-2, were also evaluated [30]. This structure was preferred, as the RMSD between the Cα atoms of the site which binds to the antibody as well as related Cα atoms of the BNT162b1-encoded spike [31], was 1.8 Å. Moreover, it is noteworthy that the S-protein structure, encoded by BNT162b1, does not comprise of a bound antibody. Thus, all in all the Omicron variant comprise of multiple mutations, which are co-evolved with alterations throughout the genetic material of the virus at an intense prevalence [11]. Furthermore, the structural evaluation portrays an exclusive position of the alterations in the Omicron variant, which might curb the antibody interaction in an individual, mediated by a previous infection or after a vaccination.

2.2.2. Escaping neutralizing antibodies pre-infection and -vaccination

The Omicron spike might escape the vaccination and infection-mediated antibodies, as suggested by the resistance offered against multiple antibodies, employed for the treatment of COVID-19. The plasma/sera obtained within the convalescence period of 2 months of, ranging from mild-type to severe form of COVID-19 disease, blocked the entrance aided by the variant spike, with about 80-fold less effectiveness, than the B.1 spike, as well as 44-fold less effectively than delta spike [12]. Out of the 17 sera tested, 9 were not able to neutralize particles consisting of Omicron spike. The samples were obtained in Germany, when the first wave of the COVID-19 infection prevailed, when neither delta nor the alpha variant dominated the population, which depicted that the antibodies produced against the infection, at the start of the pandemic, offer very less or almost negligible degree of protection against the novel Omicron variant [12]. Effective protection is observed against the COVID-19, by the mRNA-based vaccine BNT [32], and is often employed in the USA as well as Europe. Sera obtained within the period of 1–3 months, following the second dose of BNT, blocked the entrance by the variant spike, with 34-fold lesser efficacy than the B.1 spike, as well as with 12-fold lesser effectiveness than the delta spike [12]. These outcomes depict that the two BNT immunizations, that can cater > 90% protection from the severity of delta variant [33], might be significantly less efficient to combat the novel Omicron variant. Fig. 1 depicts the limited therapeutic possibility in Omicron treatment, displaying the narrow-effectiveness of antibodies and neutralization by convalescent and vaccinated sera.

2.3. Host-jumping by the Omicron variant

Humans are identified as the largest known reservoir of SARS-CoV-2. They are frequently in contact with other animal species, such as wild animals, pets, livestock animals, etc., which explains the possibility of additional variants, derived from animals, in the global populations [34]. Also, the potential of SARS-CoV-2 jumps across multiple species, which strengthens the possibility, which is well explained by Wei et al., 2021 [34], which has targeted the requirement of animal sequencing and viral surveillance, primarily those in close contact with human species. The proximal origin of the virus is questioned as a result of expeditious aggregation of alterations in the S-protein of Omicron, concerned with whether the virus originated in the human species or any other mammalian host. In the study, 45 point mutations were identified, which were acquired by Omicron variant, since the disparity from B.1.1. lineage, where the sequence of the S-protein was subjected to a positive selection of greater strength, as compared to any reported variant of SARS-CoV-2, identified to have been evolved in human hosts, which reported a prospect of host jumping. Significant differences were revealed between the molecular spectrum of alterations, which were collected by the Omicron progenitor cells and the virus which evolved in human patients. However, the data was similar to the spectra related to the virus evolved in a cellular environment in a mouse. Moreover, the alterations in the S-protein of Omicron were found to imbricate the SARS-CoV-2 alterations, identified to aid the adaptation to the host cells of mouse, specifically via elevated binding affinity of the spike protein, for the entry receptor in mouse cells. Overall, the study depicts the “jumping” of Omicron progenitor from humans to mice, followed by infection in that host, which then enters back into the humans, depicting an inter-relationship between different species, for the onset of the Omicron variant [34].

2.4. Protection against the variant spike

2.4.1. Do existing vaccines stand a chance?

Presently, it seems that the novel variant will change the immunological process of the COVID-19 patient. Some of the alterations in the Omicron are reported to compromise the viral recognition and infection attacking ability of T cells. Consequently, multiple breakthrough

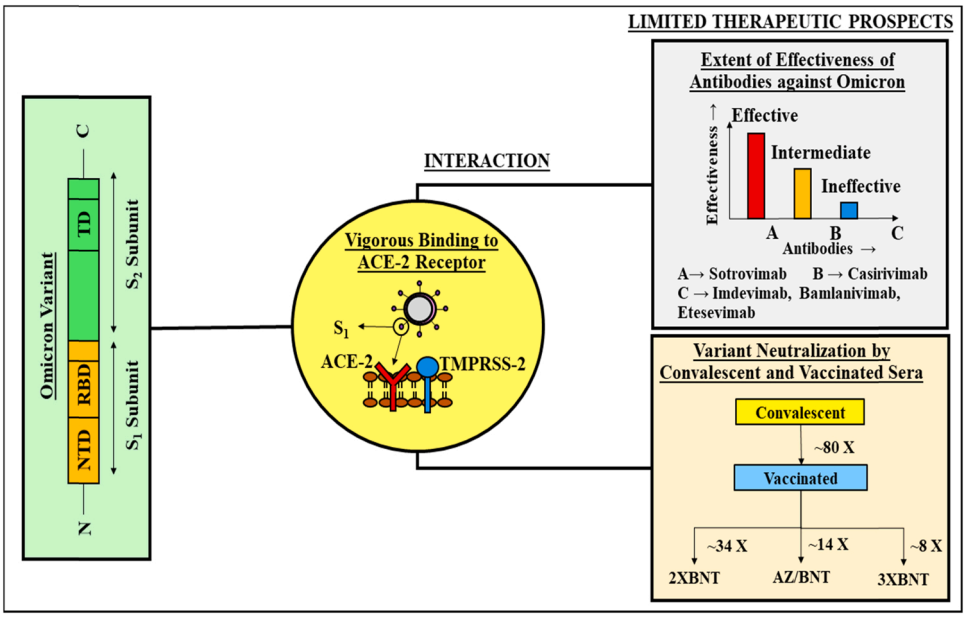

Fig. 1. Features of Omicron variant highlighting the structure and subunits; rigid plasma membrane binding via ACE2 and TMPRSS2; limited therapeutic options with reduced number of effective antibodies and antibody evasion from vaccinated sera. [ACE2 – angiotensin converting enzyme-2; TD - tetramerization domain; NTD – N-terminal domain; TMPRSS2 - Transmembrane serine protease 2].

infections can be expected in the future [35]. If the neutralizing antibodies can be circumvented by the Omicron variant, it does not neglect the probability that the immune responses mediated by the previous infection and vaccination, will aid no protection against this novel variant [35]. Curbed concentration of the neutralizing antibodies might provide protection to the individuals against the severity of the COVID-19 disease, according to the immunological, investigations. One significant parameter of the investigation would be regulation of the activity of T cells as well as natural killer cells, which might display critical significance in combating the severity of the COVID-19 disease [35]. Furthermore, quantification of the degree of protection, offered by the past infections and vaccinations, against the novel variant is another interesting research domain. Numerous antibodies can be formed by the immune system of the humans, which focus on multiple portions of the spike protein, therefore, revealing typically well performance of the vaccination, if even one part of the S-protein is altered [35]. However, the researchers are scared that this parameter might be an escape variant, as virtually all the sites that the antibodies focus upon, vary in the recently arrived Omicron variant. Initial data depicts the most infections with the novel variant are of mild nature [36]. A course (three-shot) of the vaccine of the COVID-19 disease was claimed by Pfizer and BioNTech, on 8th December, to facilitate neutralization of the novel Omicron variant, and initiated a signal that booster doses could be essential to provide protection against the Omicron variant. The neutralizing bodies were curbed by the two doses of the vaccine, however, could still provide protection against the severity of the infection, as claimed by them [37]. Similar results were displayed by another Israeli investigation, which revealed that the booster doses might be significant in the prevention from variant infection. The investigation conducted a comparative evaluation of blood samples of 20 subjects, who were administered with two vaccination doses, five to six months prior to one month before the similar number of subjects, who were administered with a booster a month before. The individuals who were incorporated with a second dose 5–6 months prior, failed to exhibit any neutralization potential against the variant [35]. Recently, a US based research [38] revealed that the efficacy of the vaccine against the Omicron-mediated symptomatic infection is expected to be greatly retarded than against the past variants. They established computational models, by using the past information on the effectiveness of the vaccines against the previous variants as well as preliminary information on the Pfizer/BioNTech vaccine, which depicted that after two mRNA vaccine doses from Moderna or Pfizer/BioNTech, the Omicron-mediated symptomatic infection is a mere 30%, unlike the 87% prevalence with the delta form of the virus. A South African data provided contradictory results [36], reporting that an array of the injections mediated by the novel variant, in Germans who were administered with full primary series of vaccination, as well as the booster with SARS-CoV-2 mRNA vaccines, exhibited intense infections with the novel variant, while in South Africa. Symptomatic COVID-19 was developed [35]. However, three mRNA doses might not be sufficient to hinder infection as well as symptomatic disease with the novel variant, as demonstrated by the mild to moderate clinical symptoms. Seven individuals were present in the reported group, with a mean age of 27.2 years, without any significant medical history. In November, on the first day, i.e., the day of arrival, a negative SARS-CoV-2 PCT test was reported in all the cases, and a complete vaccination record, even comprising of the boosters, was checked [35]. Out of those, 6 were completely vaccinated with BNT162b2 (BioNTech), and five were administered with the BNT162b2 booster in October or beginning of November, 2021. One of them receives a complete dose of mrna-1273 (Moderna), as October commences. An initial dose ChAdOx1-S (Astrazeneca) was administered to the 7th participant, after a BNT162b2 dose was incorporated for completing primary vaccination as well as a booster of the same vaccine. It was reported that they have never been subjected to the COVID-19 infection [36]. Furthermore, recently, Singapore reports have revealed the Omicron infection in two residents, after being administered with the booster dose, which portrayed its immense virulence as well as ambivalent immunological trajectories [39]. As per the predictions, these investigations support that the novel Omicron variant can evade the mRNA vaccine-induced immunity in vivo. Nonetheless, protection form the severe form of the disease might still be intact in people, who have been administered with a complete booster dose [35].

2.4.2. Can we rely on heterologous and booster vaccination?

Different strategies to enhance the development of neutralizing antibodies, with respect to BNT/BNT immunization, were explored, in order to investigate their potential in providing improved shielding to fight the novel variant [12]. Higher neutralizing antibody titers were

reported to be induced by a heterogenous vaccination, with first dose of ChAdO1- 212 nCoV-19/AZD1222 (AZ) [40] as well as second dose of BNT, unlike the analogous homologous vaccinations [41,42]. Sera obtained within a period of 1 month, post-heterologous AZ/BNT vaccination, promoted greater neutralizing activity, unlike the sera collected within the period of 3 months post-vaccination. Blockage of the cell entry, mediated by Omicron spike, from individuals vaccinated from AZ/BNT, was reported to be 14-fold less than B.1 spike, however, merely exhibiting 3-fold less effectiveness, with respect to the delta spike [12]. Furthermore, Omicron spike blockage by sera obtained within the period of 1 month of AZ/BNT vaccination, was commensurable to the blockage of delta spike via sera connected within the period of 3 months post-BNT/BNT vaccination, a time duration during which a shielding of more than 90% is catered by the vaccine, from the critical stages of the COVID-19 [12]. The protection against the infection was elevated by a third immunization with BNT, by 10-fold, in comparison to two doses of BNT [43]. Sera obtained from donors immunized with BNT/BNT/BNT, within a period of 1 month post third dose, comprised of slightly greater neutralizing titers, as sera collected in the same interval from AZ/BNT-immunized donors. Sera obtained from individuals immunized with BNT/BNT/BNT blocked the entry mediated by the S-protein of Omicron, with a reduction of 8-fold reduction in the effectiveness, in comparison with B.1 spike, as well as 2-fold reduction in efficiency in comparison to the delta spike [12]. Furthermore, blockage of S-protein of Omicron by sera obtained within the period of 1 month post immunization with BNT/BNT/BNT, was more effective than blockage of Delta spike by sera obtained within the period of 3 months after BNT/BNT vaccination. These outcomes depict that heterologous AZ/BNT as well as homologous BNT/BNT/BNT immunization might protect more effectively against the Omicron variant, in comparison with immunization with BNT/BNT (Fig. 1) [12]. The booster doses elevate the concentration of neutralising antibody, which is expected to provide defence against the potential of Omicron, as per recent reports. However, the extent of effectiveness of the doses against the viral strains, is not yet clear. People, with successive exposure to the SARS-CoV-2 spike (either via infection or booster) are more likely to exhibit the neutralising antibody activity against the novel Omicron variant [24–26]. However, a South African data revealed the insufficiency of the booster doses in the prevention of symptomatic infection, and targets the requirement to manage the supplementary non-pharmaceutical interventions [19]. These outcomes portray the requirement for modified vaccines to combat the Omicron-mediated symptomatic infection.

2.4.3. The elevated booster debate: What to do?

Till now, the booster dose has been found to cater an additional protection layer to fight the infection [44], but still, multiple questions arise, concerning the significance and need of these booster dose. Despite the elevating evidences related to the booster jabs-mediated protection against the COVID-19, the significance data associated with the impact and potential is still missing. Now, the rise of the novel Omicron variant has further disorganized the impact and role of the booster efforts on the prevalence of the pandemic [35]. Even prior to the Omicron variant, multiple healthcare institutions, at a global level, were hesitant to the large booster campaigns, despite which the rate of vaccination is limited in massive portions of the globe [45]. So far, the boosters have triggered debates over ethical topics and prioritization of limited sources of vaccines, which has brought to light the concerns of the global scientists, of vaccine imbalance, by opulent nations, who are rapidly targeting delivery of more boosters [35]. Multiple sources exist to evidently support the boosters, such as the United Kingdom (UK) [46], real-world data collected from Israel [47–49], as well as the United States of America (USA) [50], which depicted that mRNA-based vaccination boosters retard an individuals' proportion of SARS-CoV-2 contraction, as well as becoming slightly sick. Information from about 44,000 people was collected by a recent Pfizer investigation [51], which revealed that after a period of six months, vaccination-mediated protection plummet from 96.2% to 83.7%. Thereby, it can be assumed that 2 doses of the Pfizer vaccine provide a greater than 80% protection against severe form of sickness as well as mortality [35]. After 6 months of booster injections and vaccination, the protection is elevated by 10%. Thus, it would be better to deliver injections to unvaccinated people in unprivileged or developing nations, as they will receive more than 80% protection against severe disease and mortality. However, in case of full vaccinated individuals, the same booster shots would result in 10% escalation in benefit [35]. For long-term efficacy, the rise in the level of antibodies within the general population, should not be considered as evidence. The comprehensive clinical information is required to determine the necessity of the booster dose, which have been revealed to be responsible for the uncanny occurrence of pericarditis as well as myocarditis, after the incorporation of mRNA vaccination [35]. The individuals who were administered with the 2nd vaccine dose, exhibited a rate ration of 2.35 more susceptibility towards myocarditis, as compared to the ones who were unable to get the vaccine, as per an Israeli study [52]. At this point, the most significant question that arises is, does the booster benefit-to-risk ratio vary in younger and older individuals? The younger people, administered with viral vector vaccination, have been reported to exhibit thrombotic thrombocytopenia, induced by the vaccine, which creates a matter of concern [53]. However, information associated with the safety profile of 3rd-dose boosters with varying vaccines is still awaited, and thus, boosters against the novel VOC (Omicron), in the absence of reliable scientific information related to the efficacy and safety paradigm, can pose significant threats to human population [35].

2.4.4. A novel possibility:potent kinase inhibitors

A receptor tyrosine kinase, EGFR, is considered to be often altered as well as elevated in certain tumours, in response to external as well as internal stress conditions to not only enable survival, but also resistance to multiple therapy options [54]. EGFR has also been revealed to be functioning as an entry receptor for viral particles in HCV as well as influenza [55]. The aberrant STAT pathway was activated by the SARS-CoV-2 infection, which mediates an acute lung injury, elevating EGFR that results in the activation of STAT3. Furthermore, EGFR signalling can disturb the INF-γ – induced antiviral events, therefore, aiding in the infection severity [56]. A proteomics investigation revealed the phosphorylation of SARS-CoV-2 proteins, as well as the network evaluation recognized EGFR as a pivotal implication of the proteomic interactome [55]. The investigation revealed that sorafenib as well as RO5126766, can impair the entry of the viral particles, as well as infection [55]. For the mechanistic evaluation, EGFR is essentially involved in fusion of cells, during the infection mediated by the respiratory syncytial virus, estimated by other viral investigations. Also, provided the excessive EGFR/SRC kinase expression in some types of tumours, it is suggested that the cancer patient with increased concentration of EGFR/SRC kinases might be vulnerable to the SARS-CoV-2, taking in account the predicted role of the kinases in COVID-19 [35]. It is also suggested that the altered N501Y as well as other locations on RBD domain exhibit the phosphorylation ability by carrying kinases, including the EGFR [35]. Therefore, this shows that kinase inhibitors could be used as well as investigated as suitable candidates for the accurate therapy of tumour patients, with SARS-CoV-2 infection, post experimental validation. For instance, an inhibitor of EGFR, erlotinib, could be suggested for the treatment, as its complexation with INF-γ portrays antiviral potential [57]. Moreover, some herbal food materials, also comprise of natural EGFR inhibitors to provide protection against the COVID-19 – induced variants, such as foods rich in Genistein – black soybean, soybean, etc., as well as other herbal foods evaluated by the TCMSP version 2.3 database [58–60]. The nanozyme of phosphatase can be possibly produced by the processing of traditional Chinese medicinal products and herbal food, which can hinder the activity of the kinases by complexation [54]. For instance, Huangjing is already used for an anti-COVID-19 component of herbal food by clinical departments [61,

62], and should also be suggested for the protection against the viral variants. Also, the combination of EGFR inhibitor and traditional Chinese medicine, by the application of the activity of phosphatase of nanoscale Huangjing of nanozyme [60], would be reliable. Another inhibitor, capivasertib hinders viral entry via this mechanism [63,64].

2.4.5. Viral protease inhibitor: paxlovid raising new hopes

Recently, Pfizer Inc. has developed an oral antiviral drug candidate, Paxlovid (ritonavir + PF-07321332), that has been reported to deliver promising outcomes against the Omicron variant [65]. PF-07321332 is an orally bioavailable, newly developed molecular entity, which combines the advantages of both boceprevir and PF-07304814 [66,67]. This candidate is main protease inhibitor of SARS-CoV-2, and has been identified as a reliable broad-spectrum drug agent, which can be employed in the treatment of various human coronaviruses [68]. It has been revealed to exhibit strong main protease inhibitory action in Vero E6 cells with a half maximal effective concentration of 74.5 nM without cellular toxicity [68]. It can also elevate anto-SARS-CoV-2 action in mouse adapted SARS-CoV-2 MA10 model, leading to reduction in multifocal pulmonary lesions as well as load of viral particles in mouse lungs in a dose-dependent manner [68]. PF-07321332 has been reported to be safe and well-tolerated drug candidate with no adverse reactions, when administered orally in monkeys (500 mg/kg/day) and rats (1000 mg/kg/day) for a period of 14 days [68]. The favourable bioavailability as well as pharmacokinetic profile of PF-07321332 aided its clinical progression. The drug interacts with the viral enzyme via covalent linkage of Cys145 with nitrile carbon, to develop a reversible thiomidate adduct [69]. A study depicted significant susceptibility of the main protease mutants, against SARS-CoV-2 variants. There are five prevalent variants of main protease, namely T21I, K90R, G15S, L205V and L89F, owing to varying SARS-CoV-2 lineages, against all of which PF-07321332 has been depicted to exhibit potential actions, as per the enzyme kinetics. This accounts for its effectiveness in the treatment of SARS-CoV-2 omicron variant [70]. PF-07321332 was combined with ritonavir to achieve maximum potency in clinical investigations, where ritonavir retards the metabolism of PF-07321332 by blocking P450 enzymes [71]. Pfizer conducted a double blind, placebo controlled, clinical investigation to evaluate the anti-SARS-CoV-2 potential of paxlovid, where the interim results portrayed 89% decrease in the hospitalization or death cases, associated with the COVID-19, in comparison with the placebo group of patients [72]. However, it is noteworthy that paxlovid did not exhibit effectiveness at preventing death due to COVID-19, with high statistical importance [72]. Such results depicted the reliable efficacy of paxlovid as a promising candidate for COVID-19 amelioration [72]. Despite multiple investigations highlighting the potential of the drug in disease mitigation, it must be noted that excessive use or misuse of paxlovid might elevate the mutations in main protease, required for clinical resistance. Despite this, paxlovid is expected to be a safe and effective solution to the omicron spread and curb its impact [65]. As per certain studies, protection from omicron is offered as a result of previous infection with SARS-CoV-2 [73]. However, still there is a dire need to continuously look for effective candidates that might offer protection and reliability, similar to paxlovid, taking into account limited therapeutic possibilities.

2.4.6. Medicinal phytocompounds targeting omicron RBD

A study was conducted to carry out computational molecular screening against the Omicron RBD, and evaluate the binding affinity of potential drug candidates against it. Four medicinal drug compounds were brought to light by the multi-step screening of South African Natural Compounds Database (SANCDB) [74]. These compounds were SANC01032 (amentoflavone), SANC00317 (quercetin), SANC00944 (1,2,3,6-Tetragalloylglucose) and SANC00992 (luteolin), whose simulation analysis, in complex with RBD, revealed structural compactness as well as stable dynamics [74]. SANC00944 or 1,2,3,6-Tetragalloylglucose, *Ceratonia siliqua* derivative, has been revealed to exhibit significant anti-fungal, anti-cholinesterase as well as anti-oxidant actions [75]. SANC01032 or amentoflavone is an anti-helminthic flavone compound isolated from *Struthiola argentea* [76]. SANC00992 (Luteolin) and SANC00317 (Quercetin), exhibiting similar scaffolds, portray anti-tumour, anti-SARS as well as anti-microbial effects [77,78]. Furthermore, the flexibility of three loops, required for binding to Hace2 has been curbed by interaction with these drugs, as per the residual flexibility analysis data. The anti-viral efficacy of these drug candidates was portrayed by post-simulation validation of these agents, like in-silico bioactivity, binding free energy as well as prediction of dissociation constant. The complex formed by SANC01032 and RBD was reported to comprise of total free binding energy (TFBE) of – 41.88 kcal/mol, TFBE for complex formed by SANC00992 and RBD was – 29.05 kcal/mol, TFBE for complex formed by SANC00317 and RBD was – 31.03 kcal/mol and TFBE for complex formed by SANC00944 and RBD was – 46.54 [74].

2.4.7. Entry pathways are effective targets: a comparative analysis

The spike protein of SARS-CoV-2 mediated its entry into the cell by binding with ACE2 and TMPRSS2 or cathepsins [79]. The route of entry via TMPRSS2 is at the plasma membrane, which is dependent on the polybasic cleavage site between S1 and S2 subunits, as well as cleavage of spike before the release of virion from the producer cells [80]. On the contrary, the entry via cathepsin i.e., the endosomal route of entry does not require spike cleavage in producer cells [79]. A study evaluated the impact of both the form of entry pathways on omicron entry and comparatively analysed the results. The investigation results hypothesized that the concentration of TMPRSS2 Mrna levels were greater in the cells, where the omicron entry was impaired, in comparison to the delta variant entry, which led to the hypothesis that the omicron variant was deprived in terms of cells expressing TMPRSS2, in comparison to the delta variant. Furthermore, upon overexpressing the TMPRSS2, enhanced infectivity was observed for the delta variant, which revealed the fundamental need of TMPRSS2 by these variants. However, no such results were observed in case of omicron, and the use of TMPRSS2 was found to ineffective in case of omicron variant. Also, 293 T cells were used and were exhibited low endogenous levels of ACE2, but over-expressed with TMPRSS2. Elevated infection for delta variant was reported while just a small rise in omicron-mediated infection was observed. Therefore, the data suggested limited TMPRSS2 usage by the omicron variant, where the effect size is modulated by the expression concentration of ACE2 [81].

Owing to these observations, altering the TMPRSS2 usage would possible induce changes in the entry pathway of the omicron variant. To evaluate this, protease inhibitors were used to block either the plasma membrane entry pathway, i.e., TMPRSS2 blockage by camostat, or endocytic pathway, i.e., cathepsin blockage by E64D (Fig. 2). The TMPRSS2 and ACE2 – overexpressed A549 cells were infected, in the presence of camostat or E64D, where E64D (cathepsin blocker) has greater effect on omicron variant as compared delta, whereas on the other hand camostat (TMPRSS2 blocker) has a greater impact on the delta variant on the delta variant, unlike the omicron variant. This depicted that omicron is more sensitive to cathepsin inhibition, and therefore is hindered by E64D to a greater extent. Thus, unlike SARS-CoV-2 and its other variants, omicron was more affected by cathepsin or endosomal entry inhibition, which can serve as a potential target for drugs aiming to treat omicron-mediated infection [81].

3. Omicron emergence and lessons learnt from the pandemic

Grave concerns have been raised by the global community of researchers, related to Omicron emergence, on account of enormous number of alterations, unlike the past VOCs reported. Overall, 32 alterations have been observed only in the S-protein, in comparison with the 16 alterations in the greatly infectious Delta variant of the virus, besides the ones in other proteins, like NSP14 as well as NSP12, which

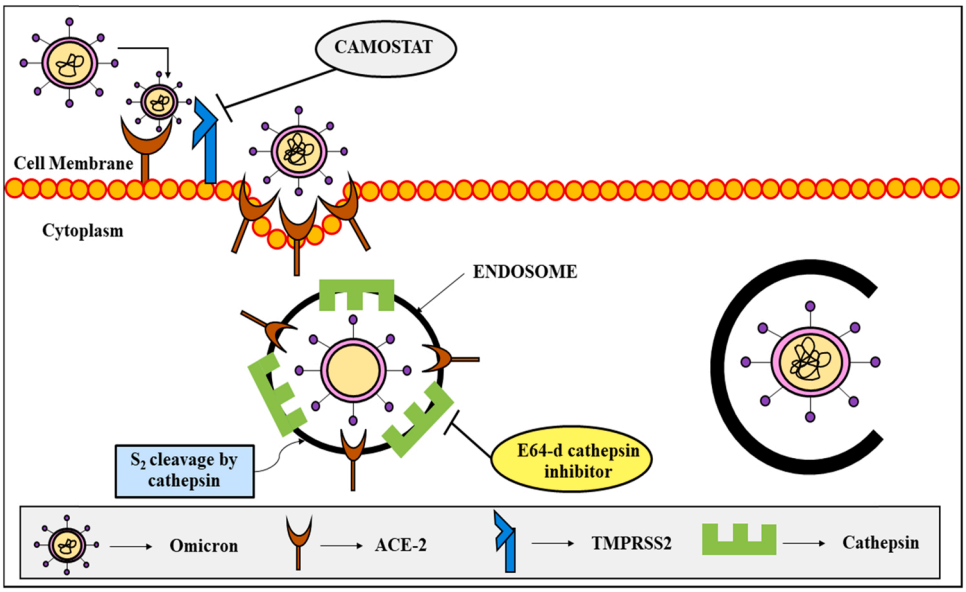

Fig. 2. Entry pathways of omicron – Plasma membrane (ACE2 and TMPRSS2) and endosomal (cathepsin), inhibited by respective blockers – camostat (TMPRSSE2 blockage) and E64-D (cathepsin blockage).

are crucial for the replications of the viral particles [82]. Furthermore, it is regarded that the novel Omicron variant could exhibit infection with the intensity thrice than the initial strain of SARS-CoV-2 [83]. Recently, an investigation has depicted that the novel Omicron variant comprises of some deletions and critical number of alterations. Certain mutations are overlapped by the alterations existing in alpha, beta, gamma and delta strains [82]. These deletions as well as alterations are well-recognised for elevating transmissibility of the virus as well as binding affinity. Furthermore, these alterations have been reported to exhibit greater probability of immune invasion or antibody escape. Even though the outcomes of other Omicron alterations have not yet been scrutinized, still there is a great equivocality as to how the complete combination would change the behaviour of the virus as well as susceptibility to both the vaccine- and naturally-induced immunity [84]. Initial evidential data depicts the common mutation shared between the alpha and the Omicron variant, i.e., P681H, which enables the easy transmission of the virus from one person to another, upon pairing with two other modifications [82]. Further, another study revealed that the cocktail of two extra mutations, N501Y and Q498R, that might elevate the binding affinity of the virus to the ACE2 receptor of the host. The N-terminal of the S-protein, which is a prominent target for neutralizing antibodies (NABs), is devoid of some amino acids [82]. The NABs are the immunological proteins which prevent viral into the host cell. Significant alterations in the target area of NABs could permit the viral particles to evade the vaccine- or naturally-induced immune response [82].

Multiple nations have made crucial adjustments in their immunization paradigm, following the emergence of Omicron variant, comprising of the recommendation for a third dose or a vaccine booster dose in big populations, to avert any after effects. The hospitalization rates of COVID-19 in England, can be retarded with the administration of the booster doses and kept under the current levels, for at least a period of two years [82]. However, the booster doses might be required every 6–12 months, in case of rapid wearing off the protection, to prevent escalation in the admissions and mortalities in the hospitals [85]. Also, it should be noted that it is more important for the individuals, who have not been administered with a single dose yet, should be immunized on a greater priority, rather than implementing the booster protocols. Variations in the rate of vaccination would not help in hindering the spread of the pandemic, as nations with ow rates are more susceptible towards the development of variants [82]. Additionally, besides the employment of booster doses, the appearance of the novel Omicron variant creates a dire need of a ban on international travels, to circumvent any disastrous after-effects. This also paves the requirement of effective border-control measures. Stringent travel restrictions should be implemented, with an effective trace system to provide sufficient amount of time to the healthcare paradigm, for the preparation of any potential excess in burden, due to rapid rise in the cases. One of the scientists of the World Health Organization (WHO) has rightly said that greater the delay in addressing the vaccination disparity, more time will the virus get to evolve, in a way that would be beyond the control of the scientists and healthcare experts [82]. There is crucial requirement for global integrated efforts, by government corporations, Pharmaceutical as well as healthcare industries, and Biotechnological companies, worldwide, to effectively regulate and manage this pandemic.

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