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REVIEW ON POTENTIAL IMPACTS OF HIGH-AFFINITY MODIFIED PROTEIN RBD-62 USAGE FOR COVID TREATMENT

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ABSTRACT

This review includes the proper aspects of designing an antiviral drug, with the application of pharmacogenomics. The binding of SARS-CoV-2 involves attachment of itself onto the ACE2 human receptor, which primarily works on the conversion of ANG II (vasoconstrictor) to ANG 1-7 (vasodilation). This binding scenario leads to the release of the viral genome into the human cell for its own functioning and replication. Therefore, the binding of viral spike protein, receptor binding domain (*RBD*) of SARS-Cov2 can be prevented by administration of protein with no therapeutic functionality with enhanced adherence to ACE2 receptor, which could initiate a potential inhibitory mechanism against the viral genomic entry. This aim is achieved by the administration of RBD-62 laboratory made high-affinity variant of SARS-CoV2 spike protein, which can inhibit the attachment of SARS-CoV2 to the ACE2 receptor, therefore inhibit the viral genome entry.

INTRODUCTION

COVID-19; the most impacted global pandemic occurred in the year 2019 to 2020. The causative agent of this is SARS-CoV2, a mutation related to both SARS-CoV and MERS-CoV (1). The widespread of this had led to a huge destruction to mankind in aspects of growth and emotional factors, leading to a remarkable decline of societal advancement (2).

SARS-CoV is an enveloped positive-sense single-stranded RNA virus from the variants of β -coronavirus, which shares 79% of its nucleotides with the SARS-CoV the causative agent for the 2002-2004 epidemic (3).

Evidence supports the existence of several human coronaviruses, including HCoV-OC43, HCoV-HKU1, and MERS-CoV, which all fall under the same genus (4). The SARS-CoV-2 virus binds to the ACE2 receptor, which is widely expressed in multiple organs such as the brain, eyes, lungs, liver, kidneys, stomach, and intestines (5). This receptor also facilitates the entry of α -coronaviruses (HCoV-NL63 and HCoV-229E) and β -coronaviruses (HCoV-OC43 and HCoV-HKU1), which are associated with mild upper respiratory tract infections (6).

STRUCTURAL INTERPREATION OF SARS-CoV2

The main functional unit of the virus consists of proteins, spike protein and genome structure. The protein that encodes

ARTICLE

ID: BTS0326021

the viral structure is divided further into 29 structural proteins and 16 non-structural proteins, this classification is based on the structural functionality difference in the proteins.

Structural proteins (29):

Encodes the structural spikes, envelope membrane and nucleocapsids.

Non- structural proteins (nsps 1 to 16):

These are the main aspects which are being focused on the designing of present antiviral drugs in markets. There are two polyproteins ppla and pplab which are translated from the RNA genome. These polyproteins are cleaved to generate the non-structural protein (Nsps) 1 to 16.

These Nsps form the replication and transcription complex (RTC), which transcribes the viral genomic and sub-genomic RNA (sgRNA) (7). These sgRNA encodes the structural and accessory proteins. Structural proteins traffic through the endoplasmic reticulum and Golgi apparatus secretory pathway, these viral genomes are packed to form budding vesicles, which are released as new virions by exocytosis.

Consideration of the RBD (receptor binding domain) is crucial which is present in the trimeric spike (S) glycoproteins of the virus (8). It helps in the adherence of viruses to the ACE2 receptor, facilitating the cell entry of viral genome. Since the RBD of SARS-CoV2 has high affinity to the human host ACE2 receptor than any other viruses, enhancing infectivity.

Finally, the major role in spreading the infection throughout the host is played by the viral genome structure. These viral genome structures form complex secondary structures, with well translation. This also

further helps in the designing of antiviral drugs.

Structural studies of viral RNA genome itself have uncovered conserved secondary structures regulating replication and translation and identified host proteins that interact with viral RNA. These are used for repurposing of drugs also directly the vaccine designing confirming the stability and immunogenicity of spike-based vaccine candidates(9,10,11).

SARS-CoV2 MUTATION

All the living things constrain Darwin's theory of '*Survival of the fittest*', viruses are no exception to this and even after being a nonfunctioning organism with mere RNA genome and proteins, it is also a constraint to the *Theory of Darwin*. The administration of vaccines makes the human body know it's opponent and it's weaknesses by immunizing our body. But, for its own survival the virus mutates itself against the functioning extremities of the vaccines, making it more virulent.

Variants of SARS-CoV2 that can acquire mutation against neutralizing antibodies include:

- B.1.351 (β)
- B.1.617.2 (δ)
- B.1.1,529 (Omicron)

After 2 doses of mRNA vaccines for COVID-19, the obvious severity of the disease has declined from ~100% in clinical trials conducted at a time when ancestral strains were predominantly in circulation to 60- 80% during Omicron wave BA.1 wave (12,13,14), a result of both waning antibody titers and virus-acquired mutations. Boosting can restore protective efficacy but the benefit of boosting beyond 3rd dose is unclear (15,16,17) and accumulating evidence suggests that

ARTICLE

ID: BTS0326021

antigenic imprinting many offsets of variant-matched boost.

TYPES AND DISTRIBUTION OF MUTATION:

Striking increases the frequency of the requirement of diverse substitutions at a critical residue W152, pointed in N-terminal domain (NTD) of spike protein. This enhances its capacity to neutralize the antibodies evasion from the body (18). NTD- exhibiting particularly high frequency of mutation requirements, suggesting an evolutionary pathway virus maintains optimal efficiency of ACE2 binding combined with flexibility facilitating the immune escape (19).

The notable mutations observed in the RBD were D614G, N501Y, E484Q, K417N/T and L452R. This modification particularly helped in the increase of transmissibility, immune escape and changes the effectiveness of the vaccine(2021).

Other recurrent mutations occur in the RNA-dependent RNA polymerase (RdRp) gene, particularly at the position 14408, this mutation influences the viral replication fidelity and drug resistance(2223).

Mutation Hotspot:

- Mutation occurs in the spike (S) protein, RNA-dependent RNA polymerase (RdRP) and other structural and non-structural proteins(2425).

Common Mutation:

- D6/4G mutation in spike protein is prevalent globally, especially in Europe and associated with increased infectivity (26).
- N501Y, T478K and RBD of the spike (2728).

Geographic Variation:

- Distinct mutation pattern and clades has have emerged in different regions, with some mutation in Europe, North America or Asia(2930).

IMPACT ON VIRUS PROPERTIES:

- 1) Transmissibility and virulence:
 - Mutation in spike protein: D614G, N501Y and T478K, leading to an increased transmissibility and altered disease severity.
- 2) Immune Escape:
 - This mostly focuses on the enhancement of the viral survival against the antibodies from vaccines, i.e., decreasing the neutralization by antibodies from vaccines.
- 3) Adaptation:
 - Human body has the best mechanism against the invasion of different types of microorganisms like virus. But on the other hand, virus also adapt themselves for the extremes of the human body conditions.

Variant Name	Key mutation	Region of Origin	Notable features
α	N501Y, D614G	UK	Increased transmissibility
β	E484K, N501Y	SA	Immune escape
γ	E484K, N501Y	Brazil/Japan	Immune escape
δ	T478K, E156del/R158G	India	High transmissibility, immune escape
omicron	Multiple Mutation	Multiple	Extensive immune escape

Table 1: Variants of SARS-CoV2

STRUCTURAL INTERPRETATION ACE2:

Full length ACE2 consists of lead peptide (residues 1-18), an N-terminal peptidase domain (PD, residue 19- 615) and a C-terminal collectrin-like domain (CLD, residues 616-768) ending with single transmembrane helix and a ~40-residue intracellular segment. Between the PD and transmembrane helix is a ferredoxin- like fold domain, termed as neck domain (residue 616 to 726) contributing to the dimerization of ACE2. Dimer formation depends on polar interaction between Gln 139 and Gln 175 on the PD dimer also contributing a weak force. Each PD accommodates one RBD when interacting with spike protein of SARS-CoV2, a key amino acid residue of ACE2 attributes to Tyr 41, Lys 353 and Arg 357 at N terminals; ASP3 and His 34 in middle bridge Gln 24 at C terminus, with Phe486 of the RBD interacting with the Met82 of ACE2 through van der Waals forces(31).

ROLE OF ACE2 IN CARDIOVASCULAR SYSTEM

Blood pressure plays a critical role in the regulation of body temperature, stability and functionality efficacy, since this is the deciding authority of the living elements. As the role seems to be crucial in human functionality, this task has been taken up by the RAAS mechanism (*Renin Angiotensin Aldosterone System*). RAAS mechanism involves the conversion of Ang I to Ang II by the action of ACE receptor leading to the vasoconstriction. The counteracting action is produced by the ACE2 receptor. This helps in the conversion of Ang II to Ang 1-7 leading to vasodilation(3233).

Main function of Ang 1-7 involves like vasodilation, vascular permeability, anti-fibrosis and anti- inflammation.

Another function of ACE2 receptor involves the conversion of Ang I to Ang 1-9, thereby activating the release of bradykinin in endothelial cells and thus have an anti-hypertrophy effect in heart. Decreases of ACE2 activity in the serum are selective cardiac systolic abnormalities in function in the development of HTN (*Hypertension*) to HF (*Heart Failure*). Absences of ACE2 enhance the sensitivity to HF, while increasing level of ACE2 prevent and reverse the phenotype of HF (34).

Degradation of Ang II to Ang 1-7 blocked by selective ACE2 inhibitors like MLN-476 D. Therefore, patients who consume ARBs or ACEIs have shown protective effect against COVID- 19, thereby reducing the risk for severe respiratory symptoms.

RBD-62 HIGH-AFFINITY PROTEIN

RBD-62 high-affinity engineered protein based on the receptor-binding domain (RBD) of the SARS-CoV2 spike protein, developed as a potential therapeutic agent to inhibit the infection by SARS-CoV2 and its variants. The unique mechanism of RBD-62 involves enhanced attachment of itself to ACE2 receptor, this is achieved by increasing the folds to 1000 thereby increasing the binding affinity to the human ACE2 receptor compared to the original viral RBD.

Viruses are prone to mutate for their own survival against the adaptation of the defensive mechanism produced by the human body. The RBD-62 includes the aspects of mutations found in the various SARS-CoV2 variants (α , β , γ) making it

ARTICLE

ID: BTS0326021

more effective method of therapy against the multiple strains (35).

RBD-62 SYNTHESIS

RBD-62 is laboratory engineered variant of receptor- binding domain (RBD) of the SARS-CoV2 spike protein, created in vitro. This was quite a challenging process for the scientist to come up with a proper high-affinity binding protein. They have tested their mutagenic libraries of RBD with human ACE2. By repeated processes of sorting them against decreasing concentration of the receptor, the high-affinity proteins are separated and the mutation S477N, E484K and N501Y, found in more transmissible SARS-CoV2 variants.

All these separations of RBD and comparison with different mutations of SARS-CoV2 against the decreasing concentration of receptor led to the identification of viral inhibition of SARS-CoV2 and its variants α , β , γ in cell culture by RBD-62.

In vivo study:

Hamsters and male rhesus monkey when administered with RBD-62, via inhalation route of administration, they had shown a potential decrease in the disease severity before or after the viral attack.

RBD-62 STRUCTURAL INTERPRETATION

The structural features and mutational impact:

- Key mutation: mutations such as S477N, E484K, N501Y, L452R and N439K in RBD increase the binding affinity to the human ACE2 and associated with higher transmissibility and immune evasion in circulating variants.
- Structural adaptation: some mutation increases rigidity in certain region of

RBD which enhancing flexibility in RBM (Receptor Binding Motif).

- Unique structural elements: RBD of SARS-CoV2 contains a flexible loop with phenylalanine F486, which penetrates a hydrophobic pocket in ACE2 strengthening the interaction compared to SARS-CoV2.

Structural analysis and functional interpretation:

- Affinity maturation: ACE2 binds up to 1000 folds increased affinity compared to wild-type RBD.
- Cryo-EM structure: 2.9 Å cryo- EM structure of RBD-62 ACE2 complex reveals the molecular basis for its enhanced binding and provide template for drug and vaccine.
- Therapeutic potential: RBD-62 can inhibit infection by multiple SARS-CoV2 variants in vitro and reduces disease severity in animal models, supporting its use as a therapeutic agent.

Glycosylation and post-translational modification:

- Expression system difference: RBDs produced in different mammalian cells (CHO vs HEK293) show distinct glycosylation pattern, affecting structural features but not significantly altering ACE2 binding or antibody recognition.
- O-glycosylation site: confirmed exclusively at T323, a previously unrecognized modification site (36).

RBD-62 RISK OVER BENEFITS

In RAAS *renin-angiotensin-aldosterone system*, the conversion of ANG II to ANG 1-7 is necessary for the vasodilation once the blood pressure retained it's normal state. If not then the ANG II binds to the AT2 receptor and leads to persistent vasoconstriction, eventually leading to extreme risk of cardiovascular complexities

ID: BTS0326021

to the patients. Therefore, this conversion plays a crucial role in the regulation of proper hemodynamic nature of an individual; this whole process is taken in control by the ACE2 receptors.

RBD of SARS-CoV2 is 1000 times folded to make RBD-62. This variant possesses thousand times enhanced binding affinity to the ACE2 receptor than the SARS-CoV2 itself. This could be a potential therapeutic methodology for preventing the viral genome entry, compared to that of vaccines or any anti-viral drugs. The concept of *survival of the fittest* plays a crucial role in all the living and non-living entities; viruses are no exception to that. They modify themselves against all the extreme conditions and lead to a more mutated version with enhanced virulence and immune escape (37).

Taking this concept into consideration, we could conclude that it is the best therapeutic innovation done in the stream of anti-viral drug discovery. But in the aspect of the high affinity modified proteins of RBD-62 tend to prevent the binding of ANGII, thereby preventing hydrolysis for vasodilation execution, leading to an increase in the risk of cardiovascular disorder.

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