

Possible Use of CRISPR-Cas13 Technology in the Pathogenesis of the SARS-CoV-2 Virus

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ABSTRACT

At present, perhaps the most important challenge of contemporary society and humanity is being experienced. A pandemic derived from the Coronavirus infection or known as COVID-19, a disease caused by SARS-Cov2. Sars-Cov2 is part of a family of coronaviruses that has direct binding to a receptor called ACE2 (human cell receptor; angiotensin-converting enzyme 2 (ACE2), receptor for agiotensin converting enzyme. This receptor is involved in a series of effects such as: regulation of arterial hypertension, diabetic nephropathy and congestive heart failure. This is due to its wide dispersion in different cells of the body. Although many alternatives have been tested, so far the only effective with significant expectation are the preventive vaccines and there is no antiviral treatment created at this time. There are many alternatives and within these it is important to explore the recent discovery of the CRISPR-Cas13 technology, which has given good results in mice and shows promise in the COVID-19 infection.

Abbreviations: ACE2: Angiotensin-Converting Enzyme 2; RBD: Receptor-Binding Domain; RTC: Replicase Transcriptase Complex; ER: Endoplasmic Reticulum; sgRNA: Sub Genomic mRNA; NHEJ: Non-Homologous End Joining; ssRNA: Single-Stranded RNA; HDR: Homology Directed Repair

Introduction

In order to start thinking about this alternative, it is important to know the mechanism of action of viruses in order to identify the attack sites of the technique. It is important to know that the infection begins in the host cell, when the virus binds to a receptor on the cell surface. The SARS-CoV-2 virus is bound by its protein (S) and the receptor for angiotensin converting enzyme 2 (ACE2). This binding directs the north of the virus in the target cell and its specificity [1]. ACE2 contributes to the regulation of blood pressure by converting angiotensin I into angiotensin [2]. The ACE2 receptor is expressed in many tissues such as the lower respiratory tract, heart, kidney, stomach, bladder, esophagus, and intestine [2]. In the lung, it is expressed in the alveoli [3]; and in the oral cavity, in epithelial cells of the tongue [4]. The SARS-CoV-2 protein (S) has

two subunits (S1 and S2). The S1 subunit is the one that interacts and binds to the ACE2 receptor through the receptor-binding domain (RBD), while the S2 subunit determines the fusion of the virus membrane with that of the host cell [5].

The virus completes its entry into the cell when the S protein is cut by the protease enzyme (TMPRSS2). This generates the separation of the RBD union of the S1 subunit with the ACE2 receptor and its fusion with the membranes, and in this way the virus is left with the door of entry open to the cell by endocytosis [6]. Once inside the cell, the translation of the viral genome and the transcription of the SARS-CoV-2 proteins begin. When it enters the cytoplasm, the nucleocapsid of the virus is released and the viral genomic RNA is released. This RNA acts as an mRNA so the viral

replicase gene can be directly transcribed [1,7]. To generate the necessary proteins of the replicase transcriptase complex (RTC), which is assembled in the endoplasmic reticulum (ER) ([1,5,7-11]. Finally, the complex (RTC) replicates and synthesizes a set of sub genomic mRNA (sgRNA) [8-11] that code for the elaboration of the main structural proteins (S), (M), (E), (N) and accessory proteins [1-5].

So, this is how RNA replication, Protein Assembly and SARS-CoV-2 Egress from the Host Cell are built. In SARS-CoV-2 replication, single-stranded RNA (+ ssRNA) serves as a template to initially synthesize a copy of single-stranded RNA (-ssRNA) [12]. And from this copy of -ssRNA, polyproteins will be produced, which will make up the RTC complex [1,8]. The RTC complex again creates a copy of the original virus genome + ssRNA from the -ssRNA template. This associates with the protein (N) forming the nucleocapsid. The structural proteins (S), (M) and (E); and accessory proteins from the endoplasmic reticulum (ER) and then, in the Golgi complex, are assembled together with the nucleocapsid to produce new viral particles, which are produced towards the plasma membrane for the release of the virus [5]. According to this final mechanism, it is where the grouped and regularly spaced short palindromic repeats (CRISPR) emerge as a therapeutic alternative, together with the endonuclease Cas, form the CRISPR / Cas complex.

This system was discovered as an immune defense mechanism present in bacteria and archaea, which incorporate DNA from pathogens, such as bacteriophages, between repeated palindromic sequences and subsequently generate an RNA called "crRNA" when transcribed. Due to its activity as an endonuclease and recognition

capacity in specific sequences, the CRISPR / Cas system has been exploited in genetic engineering to activate genes, repress them, induce point mutations and change sequences through homologous recombination. CRISPR has also been used to generate murine models of human diseases and to evaluate cell physiology through the simultaneous activation or repression of various genes [13,14]. This mechanism is made up of two factors, an endonuclease and a complementary recognition sequence. Thus: an RNA from the CRISPR sequence, called "crRNA", and the Cas endonuclease. The crRNA is in charge of directing Cas towards its complementary sequence, where Cas makes the cut.

The CRISPR sequence is composed of a leader or promoter and different spacer sequences of more or less than 40 nucleotides on average and side and side repeated sequences called palindromes with an extension of approximately 32 nucleotides Figure 1 [15]. When this mechanism enters into action in the cytoplasm, the cell recognizes a sequence known as a motif adjacent to the PAM protospacer and incorporates the nucleotides adjacent to the PAM. Figure 1. Subsequently, the crRNA is transcribed. The Cas then associates with the mature crRNA and forms the CRISPR / Cas complex. The crRNA will be the one who guides the complex towards its target through the recognition of the complementary sequence. The cuts produced by the explained mechanism are repaired by non-homologous end joining (NHEJ) and homology directed repair (HDR). As NHEJ can cause unwanted effects of unexpected mutations, it is preferred to choose HDR for this method. This CRISPR / Cas complex is a good alternative for targeted genomic editing.

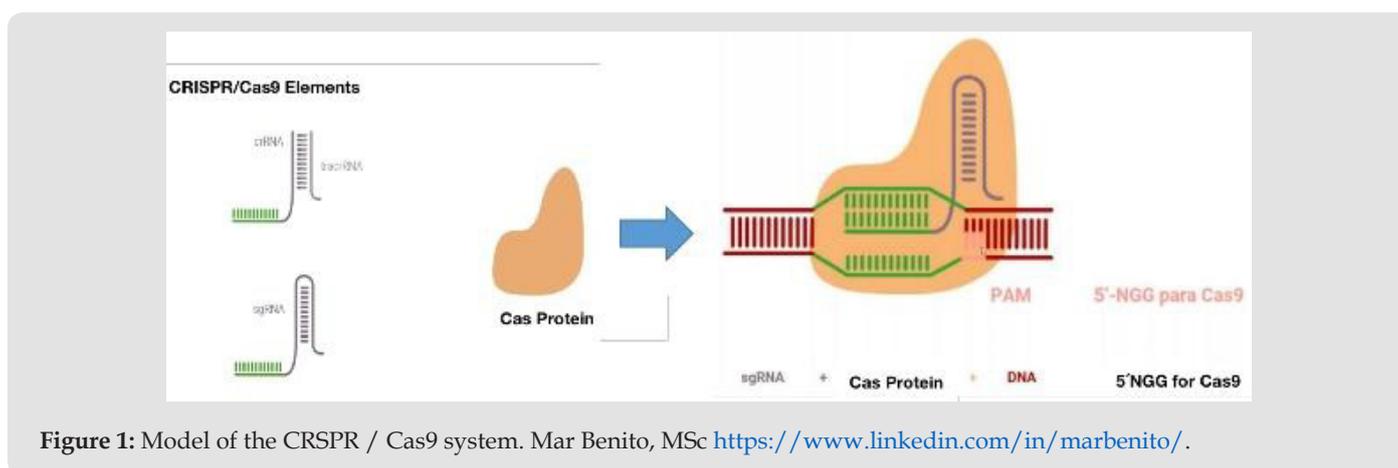


Figure 1: Model of the CRISPR / Cas9 system. Mar Benito, MSc <https://www.linkedin.com/in/marbenito/>.

The system has made it possible to insert, eliminate or generate mutations in the sequences, which leads it to target a specific sequence and induce a cut in both DNA strands [13,14]. However, this system would not be very relevant for SarsCov2, since it is an RNA virus, but within the range of possibilities and versatility of the CRISPR system, a subsystem of this complex,

known as CRISPR-Cas13, has been identified. CRISPR-Cas13 are widely distributed among Leptotrichia species. Estimating the occurrence, composition, and diversity of CRISPR-Cas13 systems in the genus Leptotrichia is particularly challenging because complete information on the entire genome is limited [16]. Cas13 is a crRNA-guided RNA-targeted effector, which has two distinct RNase sites

[17]. Not only does it highlight its unique RNA-directed ssRNA lysis activity, but Cas13a becomes a promiscuous RNase that can non-sequentially specifically cleave host cell RNA, leading to host cell or cell death latency [18,19].

Taking this information into account, this work aimed to propose an anti-COVID-19 therapeutic alternative based on the CRISPR-Cas13 system, and generate mutations in the virus, specifically in the sequence of the SARS-Cov2 protein S RBD gene that it has the function of binding to the cellular ACE2 protein and that it is the entry site of the virus for its infectivity.

Methods

First, an exhaustive review of the reported sequences of Sars-Cov2 was made, in the databases: NCBI, Google Scholar, Scopus, GeneBank, NIH. With descriptors COVID-19, CRISPR-Cas13, Gen SARS-CoV-2. The gene sequences and spike protein sequences for SARS-CoV-2 were downloaded to FASTA from GeneBank NCBI Reference Sequence: NC_045512.2. To determine the binding points of the virus to the ACE2 receptor, the sequence reported by Walls A,

et al. [20] in Cell was taken as a model. With the MEGA software [21]. The sequences were compared, and mutational analysis was carried out. In addition, mutations were made in silico that allowed the generation of stop codons that would prevent the configuration of the virus RNA and make it unfeasible for its duplication and subsequent infection. Multiple couplings of the RBD site and the ACE2 receptor were made to find binding to it and whether or not it was diminished. This was run in PyMol software [22].

Results and Discussion

SARS-CoV-2 is an RNA virus, so the best technology to attack it inside the cell is when it deposits its genetic material in the cell. The appropriate technique presented is CRISPR-Cas13, which attacks RNA and not DNA [23,24]. Based on this, the following strategy was proposed here using its CRISPR technology: This is the sequence of the Virus genome, downloaded in FASTA from GeneBank: NCBI Reference Sequence: NC_045512.2. the genome corresponding to Surface Glycoprotein S. Below, highlighted in yellow is the genome of the virus and the proteins of the virus [25].

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ATGTTTGTCTTTCTTGTCTTTATGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAA
TTACCCCTGCATACACTAATTCTTTTCACACGTGGTGTCTTTATTACCCTGACAAAGTTTTTCAGATCTCTCA
GTTTTACATTCAACTCAGGACTTGTCTTACCTTTCTTTTCCAATGTTACTTGGTTCATGCTATACAT
GTCTCTGGGACCAATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTCTTTATTTT
GCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTGGAAGACCCAG
TCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAGTCTGTGAATTTCAATTTTGTAAATGAT
CCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATCT
AGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGT
AATTTCAAAAATCTTAGGGAATTTGTGTTTAAAGAAATATTGATGGTTATTTTAAAATATATTCTAAGCAC
ACGCCTATTAATTTAGTGCCTGATCTCCCTCAGGGTTTTTTCGGCTTTAGAACCATTGGTAGATTTGCCA
ATAGGTATTAACATCACTAGGTTTCAAACCTTACTTGGCTTTACATAGAAGTTATTTGACTCCTGGTGAT
TCTTCTCAGGTTGGACAGCTGGTGTGCAGCTTATTATGTGGGTATCTTCAACCTAGGACTTTTCTA
TTAAAATATAATGAAAATGGAACCATTACAGATGCTGTAGACTGTGCACCTTGACCCTCTCTCAGAAACA
AAGTGTACGTTGAAATCCTTCACTGTAGAAAAAGGAATCTATCAAACCTTCAACTTTAGAGTCCAACCA
ACAGAATCTATTGTTAGATTTCCCTAATATTACAAACTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACC
AGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCTCTA
TATAATCCGCATCATTTTCCACTTTTAAAGTGTATGGAGTGTCTCCTACTAAATTAATGATCTCTGC
TTTACTAATGTCTATGCAGATTCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAA
ACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAAAT
TCTAACAATCTTGATTCTAAGGTTGGTGGTAAATATAATTACCTGTATAGATTGTTTAGGAAGTCTAAT
CTCAAACCTTTTGGAGAGAGATATTTCAACTGAAATCTATCAGGCCGGTAGCACACCTTGAATGGTGT
GAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTAATGGTGTGGTTACCAA

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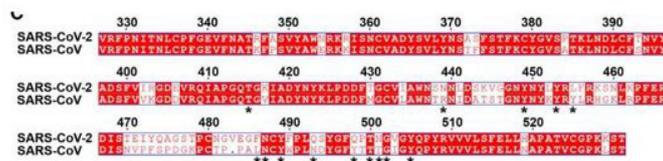
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CCATACAGAGTAGTAGTACTTTCTTTTGAACCTTCTACATGCACCAGCAACTGTTTGTGGACCTAAAAAG
TCTACTAATTTGGTTAAAAACAAATGTGTCAATTTCAACTTCAATGGTTTAAACAGGCACAGGTGTTCTT
ACTGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGCT
GTCCGTGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGTGAGTGTATA
ACACCAGGAACAAATACTTCTAACCCAGGTTGCTGTTCTTTATCAGGATGTTAACTGCACAGAAGTCCCT
GTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGTATTATTCTACAGGTTCTAATGTTTTTCAA
ACACGTGCAGGCTGTTTAAATAGGGGCTGAACATGTCAACAACCTCATATGAGTGTGACATACCCATTTGGT
GCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCTCCTCGGCGGGCACGTAGTGTAGCTAGTCAA
TCCATCATTGCTTACACTATGTCACCTTGGTGCAGAAAATTCAGTTGCTTACTCTAATAACTCTATTGCC
ATACCCACAAATTTTACTATTAGTGTACCACAGAAAATTCACCAGTGTCTATGACCAAGACATCAGTA
GATTGTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTT
TGTACACAATTAACCGTGCTTTAACTGGAATAGCTGTTGAACAAGACAAAAACCCCAAGAAGTTTTT
GCACAAGTCAACAAATTTACAAAACACCACCAATTAAGATTTTGGTGGTTTTAATTTTTTACAAAATA
TTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATCTACTTTTCAACAAAGTGACACTT
GCAGATGCTGGCTTCATCAACAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACCTCATTTGT
GCACAAAAGTTTAAACGGCCTTACTGTTTTGCCACCTTTGCTCACAGATGAAATGATTGCTCAATACACT
TCTGCACTGTTAGCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAAATACCA
TTTTGCTATGCAAATGGCTTATAGGTTTAAATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACC
AAATTGATTGCCAACCAATTTAATAGTGTATTGGCAAAATTCAGACTCACTTTCTCCACAGCAAGT
GCACTTGGAAAACCTTCAAGATGTGGTCAACCAAAATGCACAAGCTTTAAACACGCTTGTAAACAACTT
AGCTCCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTTCACGTCTTGACAAAAGTTGAGGCT
GAAGTGCAAATGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTA
ATTAGAGCTGCAGAAATCAGAGCTTCTGCTAATCTTGTGCTACTAAAAATGCAGAGTGTGTACTTGG
CAATCAAAAAGAGTTGATTTTTGTGAAAGGGCTATCATCTTATGTCCTTCCCTCAGTCAGCACCTCAT
GGTGTAGTCTTCTTGCATGTGACTTATGTCCCTGCACAAGAAAAGAAGTTCACAACCTGCTCCTGCCATT
TGTCATGATGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGT
ACACAAAGGAATTTTTATGAACCACAAATCATTACTACAGACAACACATTTGTGTCTGGTAACTGTGAT
GTTGTAATAGGAATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAG
GAGTTAGATAAATATTTTAAAGATCATAATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAA
GCTTCAGTTGTAAACATTCAAAAAGAAATGACCGCTCAATGAGGTTGCCAAGAATTTAAATGAATCT
CTCATCGATCTCCAAGAAGTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGT
TTTATAGCTGGCTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGT
TGTCTCAAGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCCAGTGCTC
AAAGGAGTCAAATTACATTACACATAA
```

This is the sequence of all the virus proteins, downloaded in FASTA from GeneBank: NCBI reference sequence: NC_045512.2. (In yellow, the genome corresponding to Surface Glycoprotein S).

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTFHAIH
 VSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIIFGTTLDSTQSLIVNATNVVIVKVEQFCND
 PFLGVYHKNKNSWMESEFRVYSSANNCTFEYVSQPFMDLEGGKQGNFKNLREFVFKNIDGYFKIYSKH
 TPINLVRDLPPQGSFALEPLVDLPIGINITRFQTLALHRSYLTGPDSSSGWTAGAAAYVGYLQPRFTFL
 LKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNAT
 RFASVYAWNKRKISNCVADYSVLYNSASFSTFKCYGVSPTKLNLDLCTNVYADSFVIRGDEVQIAPGQ
TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGV
EGFNCFYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNGLTGTGVL
 TESNKKFLFPQQFRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVP
 VAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNSYECDIPIGAGICASYQTQNTSPRRARVASQ
 SIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTTTELPSVMTKTSVDCTMYICGDSTECSNLLQYGSF
 CTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLENKVTL
 ADAGFIKQYGDCLGDIARDLCAQKFNGLTVLPLLTDEMAIQYTSALLAGTITSGWTFGAGAALQIP
 FAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLDQVNVQNAQALNTLVKQL
 SSNFGAISSVLDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLG
 QSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHGKAHFPREGVFSVNGTHWFV
 TQRNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFNHTSPDVLGDISGIN
 ASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCS
 CLKGCCSCGSCCKFDEDDSEPVKGVKLYHT

According to Walls, et al. [20]

The protein sequence has points that are the ones with the highest binding to the ACE2 receptor in the cells of our body. Marked in the figure below with asterisks [20].



When the protein and genomic sequences were compared with the MEGA software, each of the codons corresponding to these binding sites were obtained (marked in red and in bold):

ACTGGAAAGATTGCTGATTATAATTATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAAAT
 TCT**AACA**ATCTTGATTCTAAGGTTGGTGGTAAT**TATA**ATTACCTG**TAT**AGAT**TTG**TTTAGGAAGTCTAAT
 CTCAAACCTTTTGGAGAGAGATATTTCAACTGAAATCTATCAGCCGGTAGCACACCTTGTAAATGGTGT
 GAAGGT**TTTAAT**TGT**TAC**TTTCTTT**CAAT**CATATGGTTT**CA**CC**ACTAATGGT**GTTGGT**TAC**
TGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGNGYNYLYRLFRKSNLKPFFERDISTEIQAGSTPCNGV
EGFNCFYFPLQSYGFQPTNGVGY

The proposed system is for it to be removed with CRISPR-Cas13, the genome sequence of the virus surface glycoprotein S, within the cell and then replaced by the following sequence with all the binding sites changed by stop codons (the T was changed so that U converts it to RNA, from the original sequence):

UGAGGAAAGAUUGCUGAUUAUAAUUAUAAAUUACCAGAUUAUUUACAGGCUGCGUUAUAGCUUGGAAU
 UCU**UGA**AAUCUUGAUUCUAAGGUUGGUGGUAUU**UGA**AAUACCU**UGA**AGA**UGA**UUUAGGAAGUCUAAU
 CUCAAACUUUUGAGAGAGAUUUUACAACUGAAAUCUAUCAGCCGGUAGCACACCUUGUAAUGGUGUU
 GAAGGU**UGAUGA**UGU**UGA**UUUCCUUUA**UGA**UCAUAUGGUUU**UGA**CCC**UGAUGAUGA**GUUGGU**UGA**

Figure 2 shows a coupling between the RBD of the virus with the ACE2 protein. It is appreciated that when the RBD is not mutated there is better coupling with the receptor.

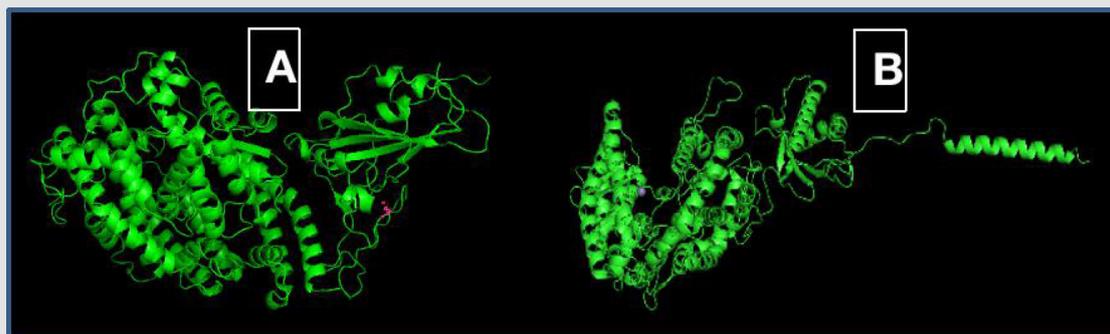


Figure 2:

- A. RBD spike protein (Right, without mutations) bound to left ACE2.
 B. RBD spike protein (Right, with mutations) bound to left ACE2. Created in PyMol.

Conclusion

In this result, some mutations were made in the possible sgRNA fragments and new mutations could be inserted as stop codons that would prevent the growth of the virus. This technique can be worked *Ex vivo* and, in this way, add to the cells strains sensitive to the action of our immune system. In this way, the virus will be unable to infect other cells. There is a truncated coupling when the protein is mutated.

Conflict of Interests

None.

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