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A Pro-Inflammatory Action of Covid-19 Causing Cytokine Storm and its role in the Development of Acute Respiratory Distress Syndrome (ARDS)

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Abstract

Severe acute respiratory syndrome (SARS-CoV-2) is a virus that has caused coronavirus disease in 2019.The virus spreading by indirectly (by droplets) or by direct contact with infected person, invades respiratory tract causing pneumonia and acute respiratory distress syndrome (ARDS).The emergence of cytokine storm in which proinflammatory cytokines including IL-6 are released upon infection with virus has been related to mortality in COVID-19 patients. COVID-19 severity is strongly influenced by proinflammatory factors particularly in older adults with chronic illness. Another consequence of virus infection is T cell dysregulation which suppress the production of interferon-gamma and impairs antiviral immunity.Thus, cytokines have a great impact in the disease incidence. The current reviewaccesses the role of cytokine storm in the severity of COVID-19 along with possible treatment interventions to alleviate it, such as the use of CRISPR/Cas13 by PAC-MAN approach, Nanotraps**,** Gallocatechin gallate(GCG)for the treatment of COVID-19.

key words: COVID-19, CRISPR/Cas13,Cytokine Storm, Gallocatechin gallate, Interleukin, Nanotraps, SARS-CoV-2

I. Introduction

Coronavirus(CoV) are enveloped, positive sense, single stranded RNA viruses that can infect humans as well as variety of other animals. SARS-CoV and MERS-CoV are two extremely pathogenic coronaviruses that has causedsevere acute respiratory syndrome (SARS) in China and middle East respiratory syndrome(MERS) in Middle East countries. SARS-CoV-2, a novel coronavirus was identified as the pathogen causing Coronavirus disease (COVID-19) in Wuhan,China, in December 2019. COVID-19 was declared a pandemic by World Health Organisation (WHO) on March 11, 2020 [1]. SARS-CoV-2 is beta coronavirus that belongs to B lineage originated from mammals specifically bats and is closely related to the SARS-CoV virus. Their genome size ranges from 26kb to 32kb.The Nucleocapsid protein (N), spike protein (S), small membrane protein (SM) and membrane glycoprotein (M) are four main structural genes, with an extra membrane glycoprotein (HE) identified in HCoVOC43 and HKU1 beta coronaviruses[2]. SARS-CoV-2 and bat coronavirus share 96% resemblance. Pneumonia was the first clinical indication of the SARS-CoV-2 associate disease COVID-19 that allowed disease surveillance [2]. More latest researches, particularly in young children's, identify gastrointestinal symptoms and asymptomatic infections. The clinical symptoms of the disease which include fever, cough, nasal congestion, weakness and upper tract respiratory infections are typically begin after less than a week in symptomatic patients. In about 75% of patients, the infection might progress to serious disease including dyspnoea and severe chest symptoms resembling pneumonia [2].

II. Infection with SARS-CoV-2 causing a Cytokine storm

SARS-CoV-2 virus particle have been found in bronchial and alveolar type II epithelial cells as per electron microscopy. During SARS-CoV-2 infection, $CD68^{+}$ macrophages, CD^{+} 20 B cells and CD^{+}T cells invade the alveolar cavity and alveoli, resulting in a cytokine stormwith adelayed interferon response. Studies have revealed that SARS-CoV-2 binds to the Angiotensin converting enzyme II (ACE2) receptor on host respiratory epithelial cells through S protein on their surface[3]. The S1 region of spike protein S binds to ACE-II receptor while the S2 subunit mediates viral and cellular membrane fusion. This mechanism generally requires S protein priming by host cell proteases, TMPRSS2 and Endosomal cysteine proteases cathepsin B and L (Cat B/L) which involve S protein cleavage at S1-S2 protein boundary or within the S2 subunit[4]. Shortly after infection, SARS-CoV-2 cause pathogenic Th1 cells to secrete pro-inflammatory cytokines including granulocyte macrophage colony stimulating factor(GM-CSF), interleukin-6 (IL-6). GM-CSF further stimulate CD14+, CD16+ inflammatory monocyte, causing them to release significant amount ofInterleukin-6(IL-6),Tumour Necrosis Factor-alpha(TNF-A) and other cytokines. As a result, high level of IL6 and TNF in COVID-19 trigger a cytokine storm [3].

III. Hyperactivation of cytokine cause ARDS

ARDS is a fatal syndrome caused by cytokine storm in which immune cells as well as non- immune cells release large quantities of pro-inflammatory cytokine such as IL6, TNFwhich harmthe host and is triggered by the upregulation of NF-kB by MyD88 pathway that involve STAT3. As aconsequence, SARS-CoV-2 infection can trigger both NF-kB and STAT3 which can then stimulate the IL6 amplifier, a mechanism for STAT3 mediated hyperactivation of NF-kB leading to the variety of inflammatory and autoimmune diseases [4]. The rapidly elevated cytokines and chemokines then attract large number of inflammatory cells including neutrophils and monocytes which invade lung tissue and cause lung injury. The delayed release of IFN alpha/beta in BALB/c mice after infection is accompanied by invasion of several pathogenic inflammatory mononuclear macrophages that obtain activating signals via the IFN a/b receptor on their surface and release more monocyte chemoattractant such as CCL2, CCL7, and CCL12 resulting in an increase in their accumulation. Increased level of pro-inflammatory cytokines such as TNF,IL-6, IL-1 and nitric oxide synthase are triggered by these macrophages which worsens the disease. These inflammatory mediators oftentriggerendothelial and epithelial cell apoptosis, destroying the pulmonary microvascular and alveolar epithelial barrier, resulting in vascular leakage and alveolar edema and ultimately hypoxia in the body, contributing to ARDS[5]

IV. The COVID-19 Cytokine storm is dominated by IL-6

IL-6 is a pleiotropic pro-inflammatory mediator produced in response to SARS-CoV-2 infection. It promotes myeloid progenitor proliferation, leukocyte activation, development of Acute phase protein such as C reactive protein (CRP). IL-6 is essential in immune responses because it promotes the differentiation of T follicular helper cells (Tfh) and along with TGF it promotes the growth of Th17 cells. IL-6 can inhibit phosphorylation of STAT-4 by activating SOCS-3 pathway, impairing the function of $CD8^+$ cytotoxic and natural killer T cells. therefore, high IL-6 level has been linked to serious lung injury. In order to avoid immune surveillance, it suppresses the role of T lymphocytes, dendritic cell and macrophages, reducing the immune system's capacity to clear infections [6].Another immune mediator, inflammasomes like NLRP3 activate caspase 1, resulting in the formation of mature IL1 and IL18 and cellular pyroptosis, which is a type of cell death in which large amount of IL-1 and IL-18 are released into extracellular matrix [7]. Pyroptosis is believed to be caused by SARS-CoV-2[8]. IL-1 is potent inducer of pro-inflammatory cytokines such as TNF-alpha and IL-6 and boosts T cell cytotoxicity. IL-6 boosts the immune response by attracting neutrophils to the lungs, where they produce leukotrienes and oxidative products that destroy the endothelium. In the presence of pro-inflammatory cytokine, IL-6 inhibits the cytolytic function of Natural killer cells, which normally target infected antigen presenting cells, triggering an inflammatory cytokine cascade amplification, stimulating macrophages to release newer pro-inflammatory cytokines, resulting in cytokine storm syndrome (CSS) [7].

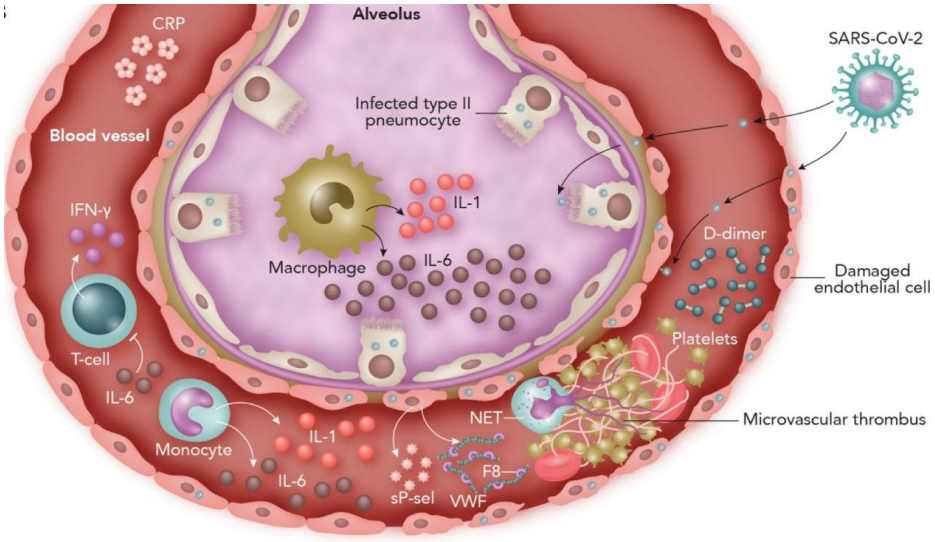

Figure 1: The emergence of a cytokine storm inside the lung's alveoli [9]

V. Role of SREBP-2 in causing a cytokine storm

SREBP's (Sterol Regulatory Element Binding Protein) are basic helix loop helix leucine zipper transcription factors that regulate gene expression in lipid cholesterol biosynthesis pathway which is known to inactivate viruses. The activation of SREBP-2 in COVID-19 patient's peripheral blood mononuclear cells was investigated and it was discovered that even though SREBP-2 expression in COVID-19 patient's plasma is increased, cholesterol level remained low by Sestrin 1(SESN1) and Proprotein Convertase Subtilisin/Kexin type 9(PCSK9). After SARS-CoV-2 infection, the SREBP-2 C-terminal fragment level rise,acting as an endotoxin, causing vascular disruption and triggering a cytokine storm by releasing IL-1beta, TNF and other cytokines hence cause severe lung inflammation [10].

VI. Dysregulation of T cells

Tcell have essential role in viral elimination, as $CD8^+$ cytotoxic T lymphocytes (CTL) secrete a variety of molecules like perforin, Granzyme, Interferon gamma (IFN-G) and $CD4^+$ helper T lymphocyte (Th) facilitate T cells and B cells in the removal of viruses from the body [11]. Type-1 IFN action is hampered in serious COVID-19 patients' peripheral mononuclear blood cells and Bal fluid, indicating decreased type-1 IFN production [12]. Hence,on SARS-CoV-2 infection the increase in cytokine such as TNF-alpha, IL-6, IL-10 reduces the number of $CD4^+$ and $CD8^+$ T cells. This indicates that these cytokines could be linked to a reduction in T cells during COVID-19. Studies have revealed that proinflammatory cytokine TNF-alpha associates with its receptor TNFR1 to promote T cell apoptosis. T cell exhibit minimal activity during infection as a result of their deprivation, which is linked to the expression of immune inhibitory factors such as PD-1 and TIM-3 on the cell surface membrane. PD-1 and TIM-3 expression continued to rise in $CD8^+$ T cells in critically ill patients. Similarly,$CD4^+$ T cells from patients in ICU level had high concentration of $TIM3^+$ cells, despite PD-1 expression in $CD4^+$ T cells being unaffected by disease progression [11].

VII. Effect on older adults

Type-2 diabetes mellitus, hypertension, cardiovascular disease, renal failure and chronic obstructive pulmonary disease all are more common in older adults [13] Immunosenescence is a progressive reduction in host's capability to mount effective immune response to pathogens where as Inflammaging is persistent rise in low-grade inflammation caused by an overactive yet inadequate warning system. In immunosenescence immune cells experience functional dysfunction such as decreased migratory, phagocytic and proliferative ability, result in weaker response and antibody production as well as inadequate clearance of foreign antigen [14].These serious illnesses make it much more inevitable that individuals infected with COVID-19 will develop more serious illness complications such as ARDS, respiratory failure and hypercoagulability. Spike protein of virus binds to ACE-2 receptor found in lung tissue, GI tract, cardiovascular and endothelial tissues. People with chronic diseases have high ACE-2 receptor expression which is thought to make virus entry far easier after infection, resulting in significant rise in innate immune system or proinflammatory signalling. Renin-angiotensin system (RAS) can also play a role in development of negative health consequences in the elderly [13]. The ACE/Angiotensin II/Angiotensin receptor type 1 (ATR1) pathway and ACE2/Angiotensin 1-7/ Mas pathway make up Renin-angiotensin system (RAS). The ACE/AngII/ATR1 pathway is linked to tissue damage, inflammation and fibrosis while ACE2/Ang 1-7/Mas pathway has anti-inflammatory and anti-fibrosis properties [15]. According to previous research, aging and chronic diseases lead to the activation of ATR 1 in mitochondria while decreasing the expression of ATR2. ATR1 stimulation enhances the release of free radical, proinflammatory cytokines such as IL-6, TNF-alpha and IFN-gamma which may intensify the inflammation and cytotoxicity induced by host upon virus infection [13]. Hence, variation in ACE2 receptor expression, oxidative stress, adipose tissue and immune senescent cell function, lack of vitamin D, decrease in autophagy and mitophagy will exacerbate the immune response in older adults, promoting the activation of cytokine storm on SARS-CoV-2 infection.[15]

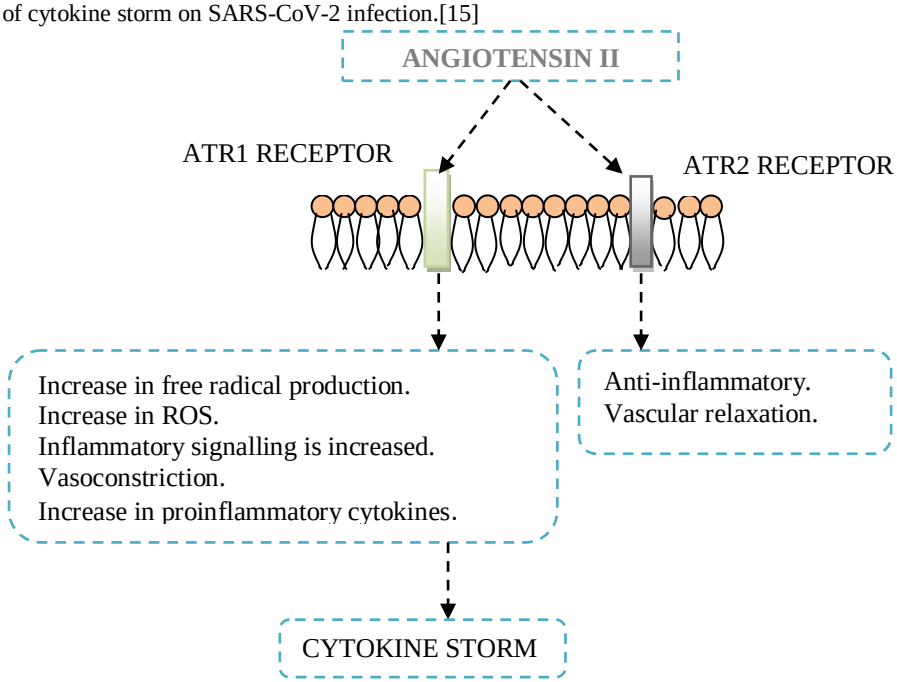

Figure 2:The role ATR1 and ATR2 in response to Angiotensin II stimulation.

VIII. Clinical Symptoms

Multiple cytokine secretion also known as cytokine release syndrome (CRS) is linked to onset of clinical symptoms[16].

Table 1: Cytokines and their clinical manifestations.

CYTOKINES	CLINICAL FEATURES
Interferon-Gamma	Fever, chills headache, dizziness, fatigue
TNF-Alpha	Fever, malaise, Fatigue, Vascular leakage, Cardiomyopathy, lung injury, Acute phase protein synthesis
IL-6	Cardiomyopathy, Vascular leakage, activate complement & coagulation cascade, Diffuse intravascular coagulation (DIC)
IL-1 Beta	Fever, Acute phase protein
IL-18	Liver damage

IX. Treatments to slow down the activity of cytokine storm

9.1. Chloroquine and its derivative Hydroxychloroquine

In the treatment of COVID-19, Chloroquine and Hydroxychloroquine have been used due to their anti-inflammatory properties. Since chloroquine and hydroxychloroquine are weak bases, they accumulate in lysosomes, an acidic organelle where they raise the endosomal/lysosomal pH thereby inhibit viral replication. Via toll like receptor signalling and cGAS stimulation of interferon genes, chloroquine and hydroxychloroquine may inhibit MHC II expression, antigen presentation and immune activation. As a result, the production of various proinflammatory cytokine involved in cytokine storm is reduced. In the treatment of COVID-19, these immunomodulatory effects can work in tandem with their antiviral effects [17].

9.2. Heparin

Heparin, a sulphated glycosaminoglycan, is one of the most commonly prescribed drugstoday.According to the studies, low molecular weight heparin (LMWH) has non-anticoagulant and anti-inflammatory properties that have potential to prevent disease progression as well as lower the level of IL-6 in plasma. Heparin works by interferingwith the receptor binding domain of SARS-CoV-2 spike S1 protein, which inhibits viral adhesion to cell surface proteoglycan, Heparin Sulphate. It alsohas anti-inflammatory properties due its ability to inhibit chemotaxis and leukocyte migration hence prevent the progression of disease [18].

9.3. Anakinra

Anakinra (ANK), a human recombinant homologue of IL-1Ra, an IL-1 antagonist receptor, inhibits both IL-1alpha and IL-1beta biological responses. ANK inhibits IL-1 activity by inhibiting type-1 cell surface receptor (IL-1 R1), which is found on the surface of majority of cells [7].

9.4. JAK inhibitors

Endocytosis allows the virus to enter into the cell by binding to ACE2 receptors. The AP2-associated protein kinase 1(AAK1) is endocytosis regulator. AAK1 inhibitors can prevent the virus infection by blocking the virus entry. Because of its relative safety and high affinity, Baricitinib, a JAK inhibitor as well as AAK1 inhibitor is used to treat COVID-19. JAK inhibitors have the ability to inhibit number of inflammatory cytokines such as INF-a, which is essential for virus control[19].

9.5. Tocilizumab

Tocilizumab is recombinant anti-human IL-6 receptor monoclonal antibody that works by blocking the binding of IL-6 to its receptor hence exerting the immunosuppressive effect that IL-6 promotes.Scientists have investigated that patients who received Tocilizumab (TCZ) has lower CRP levels. However, repeat TCZ doses may be required in COVID-19 patients who are severely ill. TCZ also demonstrated rapid regulation of extreme COVID-19 symptoms including fever and respiratory function [20].

9.6. Sputnik V

The sputnik vaccine or Gam-COVID-Vac uses a heterologous recombinant adenovirus approach that express SARS-CoV-2 spike protein having adenovirus 26 and adenovirus 5 as vectors. These engineered viruses enter the cells express the spike protein and then cease their life cycle. The virus infected cells are ultimately killed by immunity they were engineered to produce.They also don't require adjuvant and give immunity after single dose.[21].

9.7. Remdesivir

Remdesivir cansuppress SARS-CoV-2 infection in vitro by inhibiting viral RNA dependent RNA-polymerase. Scientists discovered that remdesivir may reduce the onset of more serious respiratory illness. Patients receiving low-flow oxygen showed the greatest benefit from remdesivir [22]. Coronaviruses contain"Exoribonuclease" enzyme that act as proof readers, rectifying mistakes in the RNA sequence, thereby restricting the influence of analogues, however remdesivir is able to circumvent this proofreading [23].

9.10. Gallo catechin gallate (GCG) inhibit SARS-CoV-2 replication

The Nucleocapsid (N) protein encases a viral genome, which is required for viral replication as well as for RNA transcription. The N protein produced in high quantities in infected cells, important for virion assembly and thus shielded from host cell environment [24].Liquid- liquid phase separation (LLPS) is a physiochemical process that act as key mechanism in the organisation of protein and nucleic acid into membrane less organelles which initiate biological function or reactions during variety of stresses [25]. Scientists investigate LLPS in SARS-CoV-2 N protein and find that LLPS concentrate the SARS-CoV-2 replication machinery in compartments produced by them, hence creating a mechanism for viral replication and transcription [24]. Scientists discovered that a polyphenol from green tea called Gallo catechin gallate (GCG) destabilises LLPS of N protein and inhibit SARS-CoV-2 replication by targeting N-RNA condensation, the process in which N protein interacts with viral RNA during replication and condenses into high order RNA-Protein complexes, which triggers the virion assembly and shield the viral RNA from the host RNA sensor to evade detection by host's immune surveillance mechanism. Hence GCG might be a potential therapy for COVID-19 [26].

9.11. Nano traps for SARS-CoV-2 clearance

The spike protein of SARS-CoV-2 bind to the ACE2 receptors on the host cells, allowing it to enter into the cells. Scientists discovered that Nanoparticles known as "Nano traps" fully blocked SARS-CoV-2 infection by preventing the interaction between the virus's spike protein and host cell's ACE2 receptor. Recombinant ACE2 receptors, anti-SARS-CoV-2 neutralizing antibodies and phagocytosis specific phosphatidyl serine were added to liposomal based Nano trap surfaces. Nanotrap-ACE-2 or Nanotrap-Antibody have been shown to fully inhibit pseudo typed and authentic SARS-CoV-2 viral infection in human cell lines, lung primary cells and lung organs expressing ACE-2 receptors while Phosphatidyl serine ligands on Nanotrap surfaces allow macrophages to rapidly engulf virus bound Nanotraps-ACE2 without being infected and without releasing pro-inflammatory molecules [27].

X. COVID-19 therapy with CRISPR/Cas13 by PAC-MAN approach

CRISPR is Clustered Regularly Interspaced Short Palindromic Repeats is modern genome engineering technique that consists of two parts:

a) guide RNA that is unique to target DNA or RNA sequence and
b) non-specific CRISPR linked Endonuclease protein also known as CRISPR associated protein or CAS protein.

Instead of stimulating the immune system with conventional vaccines and treatments to limit the virus entry into the cells, the CRISPR method recognises and destroys the intracellular virus genetic material and its resulting mRNA [28].

To target SARS-CoV-2, scientists develop prophylactic antiviral CRISPR in human cells (PAC-MAN) approach as a type of genomic intervention. Scientists used class 2 type VI-D CRISPR-cas13d system extracted from *Ruminococcus flavefaciens* XPD3002, a newly found RNA-guided RNA endonuclease which suppress RNA virus in human cells. Cas13d interacts with CRISPR-associated RNAs (crRNA's) which have 22-nt spacer sequence that can guide the Cas13d protein to particular RNA molecules for RNA destruction [29]. Cas13d-crRNA complex targets two highly conserved regions in SARS-CoV-2 genetic material, the RNA dependent RNA polymerase gene in open reading frame 1a/b which code for virus proliferation andthe Nucleocapsid gene at genome's 3' end [28].

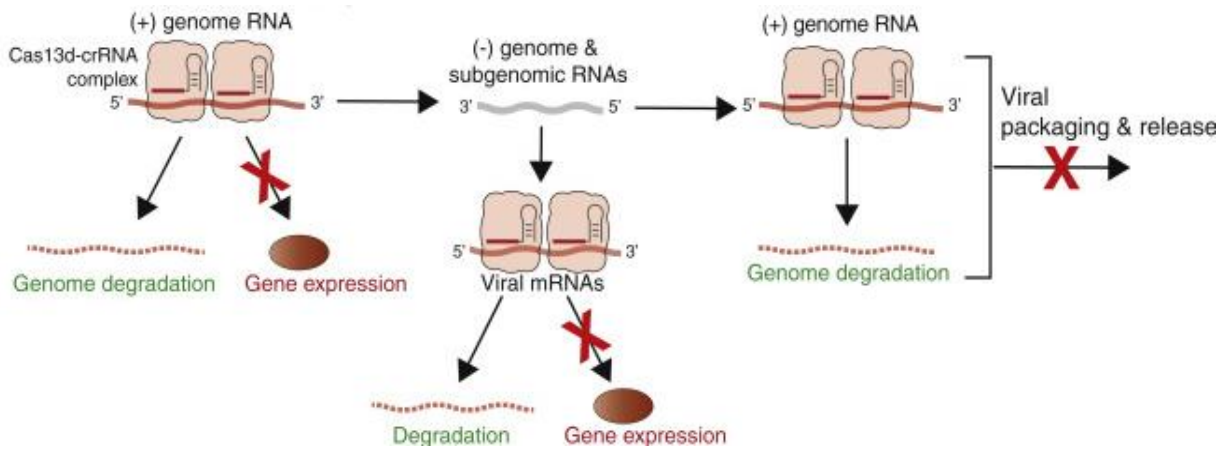

Figure 3: The PAC-MAN strategy for coronavirus inhibition by employing CRISPR-Cas13.[28]

CRISPR-Cas13 effectively restrict the viral replication by destroying viral genome template for replication and inhibit gene expression by targeting positive-sense genome and viral mRNAs.[29]. Hence, to evaluate the safety and effectiveness of CRISPR/Cas9 in eliminating COVID-19 further trials are needed [30].

XI. CONCLUSION

COVID-19, a recent and often fatal respiratory disease has quickly spread around the world. Individual to individual, educational and travel exchanges have all been curtailed. The virus has posed a threat to the countries' Health as well as medical services. Various Vaccines and therapies have been developed to combat the SARS-CoV-2 infection but still the mortality rate continues to rise day by day. The vaccines like Tocilizumab, Remdesivir have been used to combat SARS-CoV-2 but faster and more powerful methods to eradicate the virus and its variants are still required.Nano trapsare new form of nanomedicine that can be used to prevent SARS-CoV-2 infection. Another approach CRISPR/Cas9 is easy, versatile and fast approach for treating and preventing RNA viruses and the viruses that develop resistance rapidly. Further research is needed to assess the safety and efficacy of these two approaches in removing SARS-CoV-2. If these therapeutic strategiessucceed, the patients all over the world will benefit greatly. At last, there is need to conduct a thorough assessment of the world's capacity to sustain stability when face similar threats in future.

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