Genetics Section

Role of Epigenetics in Developing Therapeutic Strategies against COVID-19

RAHUL SAXENA[1], KUNAL TIWARI[2]

ABSTRACT

Epigenetics showcases an interconnection between genes and the environment. The expression or repression of genes can result from epigenetic regulatory mechanisms like Deoxyribonucleic Acid (DNA) methylation, histone modifications and chromatin remodelling. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) regulates host epigenetic machineries to mutate itself, improve its replication and increase its persistence by alienating the host's antigen-presenting molecules and modulating interferons expressing genes. The previous outbreak of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) reveals that DNA methylation by the virus plays a crucial role in the loss of antigen-presenting molecules in the host. Since these coronaviruses share an ancestorial link, it is believed that the new coronavirus acts similarly. Recent reports of increasing morbidity, mortality and persistence of Coronavirus Disease 2019 (COVID-19) points to the rapid mutation and evading of immunity of the host. Vaccines, although they have helped to prevent the pandemic but their action remains questionable with new developing variants. We explore the possibility of developing epigenetic-based drugs and vaccines and other immune modulators that are being investigated to end the present COVID-19 pandemic and open new avenues for any such pandemics in the future. Comprehensive review regarding COVID-19 was obtained from PubMed and other search engines. Insights about the COVID-19 vaccines were reported from scientific sources. Epigenetics is a crucial subject to explore for the development of therapeutic strategies against the COVID-19 virus. Epigenetic modulators that can be re-programmed to counter the replication and infection efficiency of this virus and medications, including transcription suppressors, nucleoside inhibitors, can be one of the new strategies which may have a better outcome.

INTRODUCTION

The term "epigenetics" refers to a change in the activity of DNA, Ribonucleic Acid (RNA) and proteins, without actually altering their sequences [1,2]. The epigenetic processes occur throughout the lifetime and are mainly governed by methylation, histone modification, acetylation like processes on the genes. Such alteration generates positive and negative regulatory signals, which affects the functionality and activity of the cell. Thereby, these epigenetic processes control how the gene is expressed and can modify its activity. It can therefore, be said, that apart from normal cellular processes like DNA synthesis, cell cycle, growth, development, gene expression, epigenetic processes can regulate the disease progression as well [3]. Dysregulation at the epigenetic level might cause serious pathologies, such as cancer, neurodevelopmental disorders, neurodegeneration and cognitive disability [4].

The COVID-19 pandemic, which originated from Wuhan city of Hubei province, China, in December 2019, is still a continuing threat to human health. SARS-CoV-2 is a highly contagious virus with infectivity present across a diverse human population [5] and has an increased risk of complications and mortality among ageing individuals [6].

Molecular analysis of the virus has revealed its binding domains which binds with host-specific proteins like Angiotensin-Converting Enzyme 2 (ACE2) and the transmembrane serine protease 2 (TMPRSS2). Interaction of the virus with such specific host proteins is the reason for viral entry and infectivity patterns across the global population. Moreover, it has been highlighted that multiple epigenetic changes due to this viral infection are responsible for chromatin remodelling, which impacts the host genome stability and cell homeostasis [7]. Furthermore, diverse strategies to temper host epigenetic machinery using DNA methylation, ACE2 gene methylation, and post-translational histone modification by the virus may be the cause of disease severity differences among COVID-19 infected people [8]. These epigenetic machineries can also be

used by the virus to enhance its replication and infection efficiency by regulating the host innate immune response. Therefore, tracking these epigenetic modulations may present us with novel therapeutic strategies to troubleshoot the host immune response against the viral infection [9].

Although most coronaviruses cannot regulate the host genetic sequence, but they might alter the host epigenome. New researches have focused on how the virus regulates the host epigenetic machineries to mutate itself and improve its replication and persistence [6]. Recent technological advancements have made it possible to better comprehend the landscape of these epigenetic alterations at a genome-wide level [10].

Since many viruses utilise these host epigenetic machineries to improve their establishments, SARS-CoV-2 is also assumed to follow the same strategy [11]. In present review, authors have attempted to explain the epigenetic mechanism which the new coronavirus is likely to utilise and how epigenetic vaccines can be used to boost our innate response and trigger a better response against viral infections.

LITERATURE SEARCH

The information of coronaviruses regarding epigenetics and the various vaccines developed against the COVID-19 infection was obtained from comprehensive review of literature from PubMed, Medline, ScienceDirect and other search engines, few news reports and World Health Organisation (WHO) bulletins were also referred. Insights about the COVID-19 vaccines were reported from authentic Government and Non Government sources.

EPIGENETIC MECHANISMS AND COVID-19

DNA methylation plays a vital role during the development and precise functioning of cells. A methyl group from the methyl donor S-adenosyl methionine is covalently attached to the fifth carbon of a Cytosine nucleotide, followed by a Guanine nucleotide (CpG dinucleotide). The

CpG methylation, an epigenetic mark, will be read by the replication/ transcriptional machinery similar to the non methylated cytosine. DNA methylation is recognised as an epigenetic signal by unique DNA-binding proteins which translate these signals into a cellular function. DNA methylation acts as a switching OFF/repressing or switching ON/activating epigenetic signal based on methylation pattern. As methylation levels increase, there is less transcription until the level of DNA methylation reaches the point at which the gene is switched OFF (the double helix is closed). In the absence of DNA methylation, gene transcription is allowed to occur. In other words, if all of the cells associated with a particular gene are unmethylated, they will be able to perform their programmed function. Conversely, if the gene is fully methylated, the functionality will get diminished or completely stop [12].

The viruses utilise this epigenetic mechanism to switch ON/OFF genes at multiple host gene locations. The chemical processes behind DNA methylation and histone modification also subjugate antigen-presenting molecules. These molecules are responsible for initiating host immune responses against viruses and has been highlighted in a recent study, which has concluded that DNA methylation plays a crucial role in the loss of antigen-presenting molecules after the infection by MERS-CoV [13].

Yet another study highlights that SARS-CoV and MERS-CoV hinders the host-pathogen recognition machinery and alters the interferon-stimulating-genes expression percentages by encoding unique proteins, which affect the immune signalling response [14], and the viruses develop antagonistic mechanisms to overcome the interferon-stimulating-genes effector [15]. The antiviral response of the host against the infection is mediated by the activation of interferon-stimulating genes during the viral infection, and this expression of interferons and initiation of innate immune response is regulated by specific epigenetic marks. These epigenetic marks are controlled by the epigenetic enzyme activity and chromatin remodelling (histone modification and Adenosine Triphosphate (ATP) dependent modelling to restructure nucleosome) complexes formation, which are the sites for moderation by the infected virus. With the previous knowledge about other viruses, like Human Immunodeficiency Virus (HIV-1) and Herpes Simplex Virus (HSV), which can modulate the host chromatin, it increases the chances that the coronaviruses may also act similarly [16.17].

CHROMATIN REMODELLING

Chromatins serves to pack the DNA into a much denser structure and control its function, including gene expression and other cellular processes [18]. However, they must be accessible to the RNA polymerase and other transcribing factors for genes to get expressed. The condensed state of the chromatin confines its access to the transcribing machinery, thereby restricting the gene expression. Hence, the phenomenon of the unravelling of chromatin structure into an exposed state is called chromatin remodelling. It is now clear that interaction between chromatin and the transcriptional factors act as a transcriptional regulator. Histone modifications are responsible for triggering the chromatin for further modifications. Post-translational modifications are made to the amino-terminal tails of histone proteins, resulting in a dynamic interplay between histone and DNA modifications, as well as the combination possibilities for gene regulation. The process leading to structural changes in chromatin can be interpreted into two parts. The first is the breaking of interactions between nucleosomes, second, where various protein factors are recruited to unravel the nucleosome [19]. Moreover, multiple histone-modifying enzymes are also involved with the process, namely, serine/threonine kinases, methyltransferase, acetyltransferases, proline isomerases and ubiquitin ligases [19]. Such enzymes are responsible for chromatin modifications.

These major epigenetic machineries not only control the host immune response but can also ensure their operational control. SARS-CoV virus has shown a strong association with RNA modifications like N6-methyladenosine (m6A) and N6,2′-O-dimethyladenosine (m6Am)

modifications (m6A/m) which have been found to play essential roles in the viral life cycle [11]. These modifications also affect the structure and replicating ability of the virus along with the host's innate recognition and immune response processes. The m6A RNA methylation is the most abundant epi-transcriptomic modification of eukaryotic mRNAs and has been detected on cellular and viral transcripts, regulating numerous biological processes, including viral infection [20,21].

Coronaviruses like SARS-CoV and MERS-CoV possess the ability to mediate epigenetic modifications by alienating the host's antigen-presenting molecules and modulating interferons expressing genes [22,13]. This process can be understood by analysing DNA methylation signatures among various immune and blood cells at different time intervals. Evaluating the past, during, and post-effects of infection by a coronavirus in the human population could also explain the variation in severity of this disease [23]. The vulnerability of senior citizens to SARS-CoV-2 infection may also be related to the ageing epigenome constitution leading to easy entry of the virus into the host [24]. This viral entry process is guided by the interaction of viruses' Receptor-Binding Domain (RBD) part of spike protein with the human ACE228 and Dipeptidyl-Peptidase 4 (DPP4) receptors [25].

PREVENTION AND TREATMENT STRATEGIES AGAINST COVID-19

The first strategy adopted for COVID-19 treatment appeared very promising in the beginning. Several antiviral drugs like Remdesevir, Favipiravir, Lopinavir/Ritonavir or Umifenovir were administered either alone or in combination with antimalarial chloroquine/ hydroxychloroquine chemicals [11]. Currently, no specific drug is available for the treatment, but previous drugs have been repurposed based on their potential to either negate virus, reduce lung inflammation or disease symptoms. Nevertheless, WHO has put forward several clinical trials for the use of hydroxychloroquine, Remdesevir with or without Lopinavir in combination with Interferon (IFN) beta 1a against COVID-19 [26].

Other combination strategies included biologicals like convalescent plasma or mesenchymal stem cells and mesenchymal stem cell-derived exosomes. Chinese traditional medicines and other supplements with vitamin C and D were also considered [27-30]. These drugs target transcription, viral translation, protease, and autophagy. Therefore, it has proven to bring relief in suffering patients of SARS-CoV-2 infection. Although these drugs targets all the reverent checkpoints in the SARS-CoV-2 life cycle, mixed results were reported, which included side-effects as well [31]. These drugs were selected and re-purposed for the use against SARS-CoV-2 based on past experiences during SARS infections [32].

The second approach to treat COVID-19 disease was convalescent plasma therapy, where the plasma was extracted from COVID-19 recovered individuals. The convalescent plasma approach showed the best results in terms of recovery [33].

Yet another approach taken was of immunotherapies. Since most immunotherapies are still under clinical trials, results for their use against SARS-CoV-2 have yet to be pressed. Due to the reason that vaccines need more time to design, test and manufacture, the approaches mentioned above were the most promising in the hours of crisis. Upon completion of the vaccines clinical trials, the ones which performed greatly in terms of safety and efficacy during the phase-3 clinical trials were given the emergency authorisation for human use and is listed under the Emergency Use Listing by the WHO. These were COVISHIELD (ChAdOx1 nCoV-19) which is manufactured by AstraZeneca and Serum Institute of India, COVAXIN by Bharat Biotech [waiting for approval by WHO for Emergency Use Listing], SPUTNIK- V by Gamaleya and Dr Reddy's, mRNA-1273 by Moderna, mRNA-BNT162b2 by BioINTech and Pfizer, Non replicating viral vector vaccine Janssen owned by Johnson and Johnson and inactivated SinoVac and Sinopharm [34]. [Table/Fig-1] shows some of the COVID-19 Vaccines [35-52].

EPIGENETIC APPROACHES TO COVID-19 MANAGEMENT

As DNA methylation and histone modification governs ACE2 regulation, the epigenetic enzymes that control these processes, they become the potential targets for modulating the host immune system. These enzymes include DNA methyltransferase 1, histone acetyltransferase 1 (HAT1), histone deacetylase 2 (HDAC2), and lysine demethylase 5B (KDM5B). Inhibitors of these enzymes can therefore be re-purposed to treat coronaviruses infection. DNMT1 inhibitors, e.g., Azacitidine, HAT1 inhibitors, such as anacardic acid, and HDAC2 inhibitors, such as valproic acid, can be the potential therapeutic chemicals for this approach [11].

Name of vaccine	Manufacturer	Country name	Type of vaccine	Efficacy	Shelf life (Storage)	Route	Number of doses	Schedule	Adverse effect following immunisation	Any other instruction	Reference
ChAdOx1 nCoV-19, COVISHIELD	Astra Zeneca	United Kingdom	Viral vector encoding a spike protein	81% symptomatic	6 months stored in a refrigerator between 2 to 8°C	Intramuscular injectable	2	12-16 weeks apart	Injection site tenderness/pain, headache, fatigue, myalgia, arthralgia, malaise, pyrexia, chills, nausea/vomiting	After first opening, use the vial within 6 hours when stored at room temperature.	35
	Serum Institute of India	India									
COVAXIN	Bharat Biotech	India	Whole-virion inactivated	81% symptomatic	24 months stored in a refrigerator between 2 to 8°C	Intramuscular injectable	2	4 weeks apart	Injection site tenderness/pain, headache, fatigue, myalgia, arthralgia, malaise, pyrexia, chills, nausea/ vomiting	Use of Chloroquine and Corticosteroids may impair antibody response	36
SPUTNIK- V	Gamaleya	Russia	Viral vector encoding a spike protein	91% symptomatic	2 months stored in a refrigerator between 2 to 8°C	Intramuscular injectable	2	3 weeks apart	Pain and swelling at the injection site, flu like illness, fever, fatigue, body aches, headache	Shake well, before use	37
	Dr Reddy's	India									
Spikevax, mRNA-1273, CX-024414	Moderna TX, Inc.	United States	Recombinant mRNA encoding a spike protein	94.1% symptomatic	7 months stored in a freezer at -25°C to -15°C	Intramuscular injection	2	4 weeks apart	Pain, Redness, Swelling, Tiredness, Headache, Muscle pain Chills, Fever, Nausea	Thawed prior to administration. Vaccine in the dosing syringe prior to administration. The white to off-white suspension may contain white or translucent product related particulates.	38
mRNA-BNT162b2	BioNTech and Pfizer	Germany and United States	Recombinant mRNA encoding a spike protein	95% symptomatic	9 months stored in a freezer at -80°C to -60°C	Intramuscular injection	2	3 week apart	Soreness, fatigue, myalgia, headache, chills, fever, joint pain, nausea, muscle spasm, sweating, dizziness, flushing, feelings of relief, brain fogging, anorexia, localised swelling, decreased sleep quality, itching, tingling, diarrhoea, nasal stuffiness and palpitations	Prior to dilution and after thawing, gently invert the vial 10 times to mix; do not shake	39
JNJ-78436735	Johnson & Johnson	United States	Viral vector encoding a spike protein	66% symptomatic	4.5 months stored in a refrigerator between 2 to 8°C	Intramuscular injection	1	-	Risk of a rare adverse event called Thrombosis with Thrombocytopenia Syndrome (TTS)	There should be a minimum interval of 14 days between the administration of this vaccine and any other vaccine against other health conditions	40
Sinopharm BBIBP-CorV	China National Pharmaceutical Group Co., Ltd.	China	Whole-virion inactivated	79% symptomatic	24 months stored in a refrigerator between 2 to 8°C	Intramuscular injection	2	21-28 days apart	Dizziness, fatigue, headache, nausea, vomiting, fever and allergic dermatitis	The first dose and second dose should be the same vaccine. However, if severe allergic reactions develop after the first dose, other alternative vaccines might be considered. Once administrative guideline for vaccine interchangeability becomes conclusive, the recommendations might be changed in accordance with standard guidelines.	41

Vaccine	Manufacturer	Country	Type of vaccine	Efficacy	Storage	Route	Doses	Dose interval	Adverse effects	Remarks	References
CoronaVac	Sinovac Biotech Ltd.	China	Whole-virion inactivated	66.6% against symptomatic	24 months stored in a refrigerator between 2 to 8°C	Intramuscular injection	2	4 weeks apart	Injection site reactions fatigue, diarrhoea, and muscle pain	Avoid exposing CoronaVac to the disinfectant during use. Do not freeze. It should be administered immediately after opening the vial. If the second dose is administered less than 2 weeks after the first, the dose does not need to be repeated.	42,43
ZyCoV-D	Cadila Healthcare Limited	India	Plasmid DNA	67% against symptomatic	3 months stored in a refrigerator between 2 to 8°C	Intradermal pharmajet	3	28 days apart	Fever, headache	Three doses of the ZyCoV-D vaccine will be administered on day 0, day 28 and day 56. Each of the three doses will be given in two shots on each arm, right and left; for an individual to be fully vaccinated by ZyCoV-D, one would have to be injected with six shots of the vaccine.	44,45
INO-4800	Inovio Pharmaceuticals	United States	DNA encoding spike protein	91% symptomatic	>3 months at 8°C ; five-year projected shelf life in a refrigerator between 2 to 8°C	Intradermal injection	2	28 days apart	Fatigue, headache, and muscle pain.	Delivered intradermally followed by electroporation	46,47
Nuvaxovid, Covovax, NVX-CoV2373	Novavax, Inc	United States	Recombinant nanoparticle vaccine (protein subunit vaccine and a virus-like particle vaccine)	80% symptomatic	Storage at 2°C to 8°C	Intramuscular injection	2	21 days	Fatigue, headache, and muscle pain.	Each injection includes many spike nanoparticles, along with a compound extracted from the soapbark .	48,49,50
CoviVac	Chumakov centre	Russia	Whole-virion inactivated	80% symptomatic	Storage at 2°C to 8°C	Intranasal	2	14 days apart	Fatigue, headache, and muscle pain	Vaccine administered to a person via the nose and does not	51,52

[Table/Fig-1]: Overview of the COVID-19 vaccines based on manufacturer, country of origin, type of vaccine, its doses and adverse effects etc [35-52].

However, the drug dosages for treating COVID-19 may exhibit severe side-effects like, mild tubular and interstitial haemorrhages and disruption of capillaries, accumulation of mucoproteins in renal tubules, inhibition of gene transcription and enhanced proinflammatory transcription leading to cell death and apoptosis [27,53,54]. Epigenetic drugs based on their anti-inflammatory action for treatment of cancer, by moderating host cellular epigenetic machinery can be a promising antiviral therapy, since the virus multiplication and persistence relies on the same machinery [55,56].

Recent studies also reveal that the primary cause of death in SARS-CoV-2 infection was the uncontrolled production of soluble markers of inflammation, referred to as cytokine storm. Decitabine, a nucleoside-based DNMT inhibitor, which is found to reduce inflammation and IFN response in macrophages by inhibiting DNA methylation, could be a promising therapy to prevent complication and mortality in COVID-19 patients [Table/Fig-2] [31,57]. It has been recently included in a therapeutic trial for COVID-19 pneumonia-Acute Respiratory Distress Syndrome (ARDS) [58].

Natural killer cells and lung innate lymphoid cell group 2 govern the long-term strengthening of the innate immune response through epigenetic processes, according to a study on innate immune cells [59,60]. Upon exposure to viral infection, these cells undergo metabolic, mitochondrial and epigenetic reprogramming. As a result, the improved immune response to secondary heterologous

infection develops the memory phenotype [61]. The research also points to the role of β-glucan in immunological dysregulation and cytokine storm in COVID-19 afflicted people. Their studies observed that β-glucan-driven memory-based immunity also determines some epigenetic changes and that it could represent a valuable target for COVID-19 treatment [61].

In addition, a few studies suggest that COVID-19 patients should consume vitamins and other natural products to boost their immunity and minimise any inflammatory response [62-64]. Vitamin D and quercetin may reduce COVID-19 disease severity by decreasing the expression of ACE2 and its possible function in regulating the cytokine storm linked to COVID-19 patients mortality [63,65].

It's worth noting that the Polycomb Repressive Complex 2 (PRC2), which controls transcription suppression via H3K27me3 enrichment at particular IFN-stimulated genes, might potentially be another target for research. PRC2 inhibitors are presently being tested for cancer treatment and thus, can be repurposed for the use against COVID-19 infection [66]. Despite the fact that no epigenetic medications are currently available, various methods are being investigated which may have a better outcome, few of those strategies are discussed below.

1. SARS-CoV-2 treatment with drugs using histone modification:
A recent study [58] on immune epigenetics has concluded that coronaviruses have a persistent need to modify histone protein
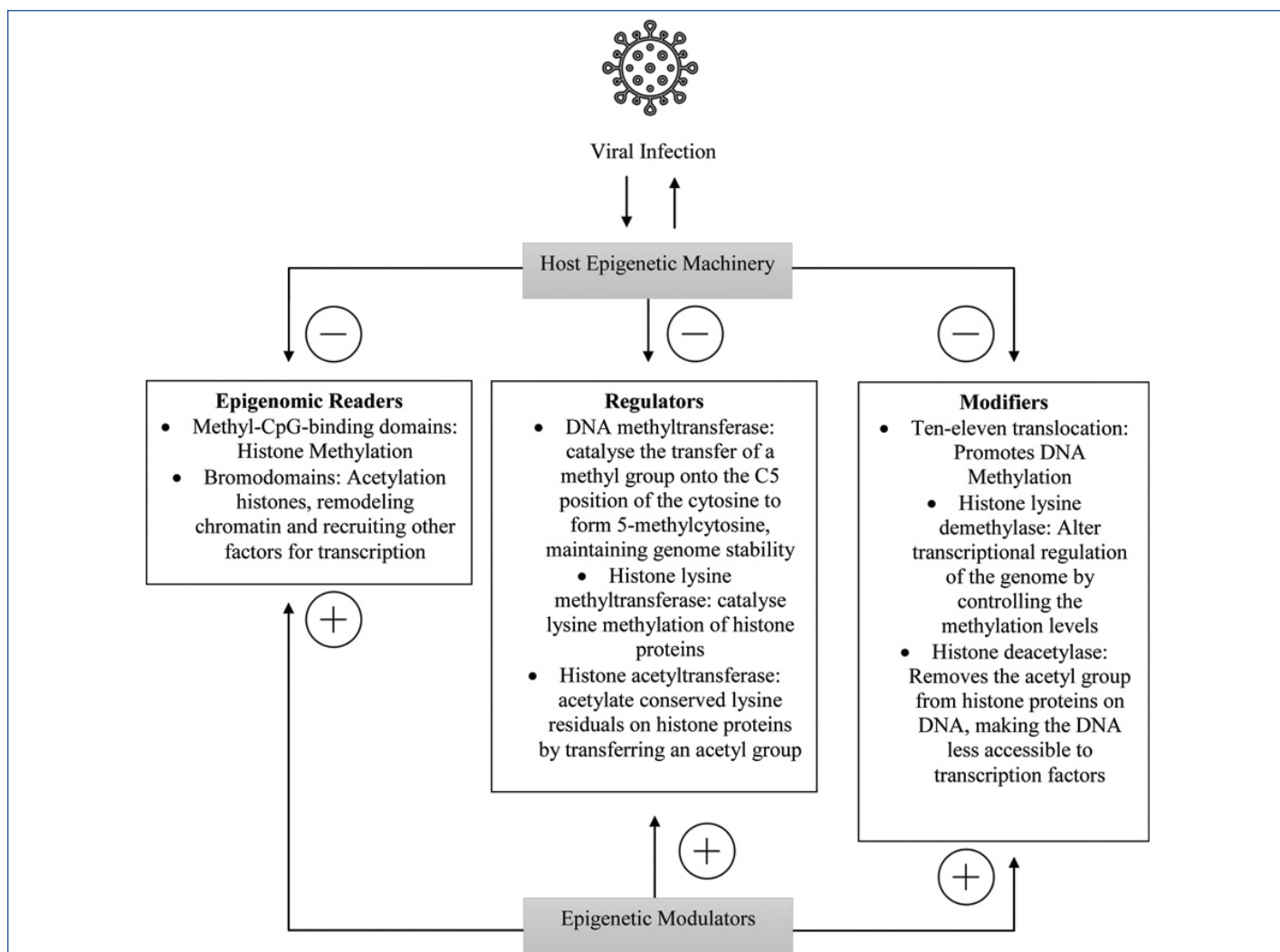
[Table/Fig-2]: Interaction between host epigenetic machinery and epigenetic modulators during a viral infection.
(+): Host machinery action; (-): Viral effect

to improve its establishment. The strategy the virus engages in overpowering the human epigenetic machinery is histone mimicry. This is done by replacing a part of the host's epigenetic machinery with a viral protein, thereby causing gene silencing and enhanced inflammatory response [67]. Hence, these foreign proteins can be a potential target for decreasing the viral load and decreasing the burden on the host's immune system. Since the histone mimicry approach is still left to be uncovered for most viruses, the possibility of a generalised epigenetic drug remains untouched [68].

2. Awakening innate immunity sensors to fight virus: Even though the development of vaccines against the SARS-CoV-2 has dramatically reduced the necessity of finding any other preventive strategy but their efficacy against the developing mutant variants is falling with the emergence of new variants, which is of concern today in the fight against the COVID-19. The vaccines usually trigger only the adaptive immune system to fight against the COVID-19 virus. Still, the possibility of improving vaccine adjuvants that acts as immune potentiators by modifying the body's innate immune system can help complement the actions of vaccine [69].

To treat SARS-CoV-2, immunomodulation by Toll-Like Receptor 5 (TLR5) activation, which is essential in innate immune sensing, might be a novel strategy. In motile gram positive and gram negative bacteria, these TLR recognise the structural protein of the flagellum [70]. Therefore, it can be hypothesised that flagellin, which is a disguise of SARS-CoV-2, may signal similar to a bacterial infection but instead triggers an innate antiviral immune response [34]. Similar approaches are being trialled for influenza viruses [71]. Recent research [72] on m6A methylation conducted in light of this notion is an effective strategy that might lead to the first epigenetic based drugs and vaccines that targets both innate and adaptive immune

responses [72]. There are other potential areas still left to explore for the development of any other preventive mechanism as in future, other options may be considered as well.

OTHER NOVEL APPROACHES TO COVID-19 MANAGEMENT

Probiotics as Immunostimulants

Researches suggests that the human microbiome is linked with both innate and adaptive immunity. The human pharyngeal microbiome, which thrives at the nexus of the digestive and respiratory systems, is critical in avoiding respiratory infections in normal physiology. Since respiratory inflammation or ARDS is one of the hallmarks of COVID-19 disease, the pharyngeal microbiome may interact effectively with the local epithelial and immune cells, forming a unique micro-ecological system capable of eliciting host immunological responses to neutralise invading viruses [67].

Previously, many studies [73,74] were conducted on the microbiome-manipulation or probiotic activity in gastrointestinal diseases. Microbiomes with similar activity in the respiratory region of the body may play a fundamental role in the treatment of COVID-19 disease. There is evidence that the microbiome community of the nose and throat is associated with influenza susceptibility [75], and identifying stable microbiomes in these regions may protect us against SARS-CoV-2 infection, decreasing the need for a strain-specific vaccine. Knowledge of microbiomes in numerous parts of the body might lead to the development of alternative therapies that can be tailored to treat SARS-CoV-2 and other similar infections. [76].

Probiotic nasal spray in an animal study was found to decrease symptoms of illness and viral titres in mouse models, improving their

survival [62]. Recently studies [77,78] have focused on the effects of various *Lactobacillus* strains on viral infections. These nasal probiotics have proven to induce the expression of viral defence genes such as IFN-beta, Interleukin (IL)-12, and IL-10. They have also been reported to inhibit respiratory tract influenza infection via regulating the microbiota-controlled Toll like Receptors-7 (TLR) signalling pathway [79]. The mechanism behind protection from disease in these mouse models might be due to the direct probiotic viral interaction or by immunomodulation through these probiotics. Evidence from in-vitro models supports this concept, *Lactobacilli* binds and inhibits Vesicular Stomatitis Virus (VSV) strains in a cell culture model [67]. With the promising results obtained from these studies, performing similar tests against the COVID-19 virus might prove beneficial.

Probiotics present us with other advantages as well; they secrete a range of antiviral metabolites, like violacein, that can kill viruses or inhibit replication. Additionally, other members of the microbiome family can also reduce initial viral titres in the airways and, therefore, can lead to a milder infection [80]. These advantages project us with a prevention strategy against the cytokine storm, one of the hallmarks of COVID-19.

Nano-medicine
Another potential field to approach for COVID-19 management is using aerosol inhalation of therapeutic nano-medicine agents. Studies are focusing on the use of Mesenchymal Stem Cell (MSC)-derived exosome and interferon-beta inhalators as therapeutics. Results from clinical trials in this field could provide more insights into this approach [81].

Host-dependent RNA Editing of SARS-CoV-2 Genome
RNA editing by host deaminases is an innate restriction process to counter viral infection [82]. However, RNA editing directly affects the genetic information of the viral genome; therefore, it may demise or fuel the virus evolution. The human Adenosine Deaminase Acting on RNA1 (ADAR1) is an RNA-binding protein, which functions through post-transcriptional modification of mRNA transcripts and is considered as a master regulator of cytoplasmic innate immunity. ADAR1 catalyses the deamination of adenosine to inosine in double-stranded RNA. It can exert either antiviral or pro-viral effects depending upon the infecting virus. For example, hyper editing of HCV and Lymphocytic Choriomeningitis Virus (LCMV) viral genomes lead to antiviral effects, while ADAR1 editing of influenza A RNA enhances viral protein expression [83]. In the case of COVID-19, ADAR contributes to the evasion of type-I-interferon responses [84]. Inhibitors of ADAR1 leads to viral inhibition by enhanced interferon stimulation in primary macrophages [85]. Therefore, this can be another strategy to boost antiviral response in viruses that trigger suboptimal interferon responses, as seen during SARS-CoV-2 infection. However, mutational analysis of genomes from different viral and human transcripts reveals mutational patterns with reduced ADAR signatures [86]. In contrast, after 24 hours post-infection of SARS-CoV-2, higher levels of A-to-I editing were recorded, although accounting for <1% of sites.

DISCUSSION
The epigenetic process occurs throughout the life and is essential for altering positive and negative regulatory signals, which affects the functionality of any cell [3]. SARS-CoV-2 outbreak has been one of the deadliest threats to human health across the globe. One of the leading causes of death among COVID-19 patients is the development of cytokine storms leading to tissue damage and multiorgan failure, thus death. Studies have revealed that COVID-19 virus modulates the host epigenetic machinery to regulate the expression of many proinflammatory cytokines, including TNF-α, and to maintain its virulence by evading the immunity. Dysregulation of the epigenetic machinery of the host by methylation and post-translational histone modification by the

COVID-19 virus might be responsible for a diverse spectrum of diseases and their severity. [8,11].

Response of host immune cells against infection is mediated by activation of antiviral genes. As the virus hinders the host immune response by DNA methylation (switch OFF) of these genes at multiple host gene locations [14], the first strategy could be the unmethylation of genes in the affected cell, which may allow the gene transcription in the cell to perform their programmed function. [12]. Epigenetic enzyme activity and chromatin remodelling by histone-modifying enzymes and ATP dependent restructuring of nucleosome [16] are moderated by the virus [11]. Preventing the unravelling of chromatic structure by the virus can be yet another strategy to prevent infection. Since epigenetic modulating the DNA methylation and histone modification by using the regulatory enzymes by the virus is the potential targets for modulation host immune response, inhibitors of these enzymes can therefore be re-purposed to treat coronaviruses infection. Also, epigenetic drugs for the treatment of cancer can also be a good candidate as a broad-spectrum viral action and inflammatory function [54].

Based on the fact that natural killer cells and lymphoid cells strengthened by epigenetic process on viral interaction can give insight to β-glucan-driven memory-based immunity that it could represent a valuable target for COVID-19 treatment [60]. PRC2, which controls transcription suppression via H3K27me3 enrichment at particular IFN-stimulated genes, might potentially be another target for research. The persistence of the virus based on histone protein mimicry by the virus by developing viral proteins can be a potential target for decreasing the viral load and decreasing the burden on the host's immune system [58].

The development of drugs and vaccines requires knowledge of viral genome mapping from a global perspective so that the function of epigenetics in the viral infection can be further investigated using this genomic information of methylation and chromatin structure. Investigations concerning the epigenetic causes of disease severity might present us with new strategies against this virus. Redesigning antiviral drugs that target specific epigenetic modulators and new chromatin-based therapies against viruses like SARS-CoV-2 may decrease the disease severity [87].

At the same time, it is equally important to develop strategies for prevention against the infection by making the innate immune response more stronger by identifying protein-protein interaction to map host-virus interactions and using small RNA molecules that enable long-term epi-antiviral control. The unique microecological system of the body may be investigated as a promising therapy for enhancing and eliciting host immunological responses to neutralise the invading viruses by immunomodulation [33]. In this regard, it has been remarkable to see all the scientific communities worldwide collaborating to expedite the newer strategies in vaccine and antiviral drug development. Tracking these epigenetic modulations might present to us with novel therapies to develop strategies to regulate host immune response against the virus [9].

CONCLUSION(S)
Epigenetics has a significant role in the persistence and progression of COVID-19 disease. Epigenetic modulators and epigenetic medications, including transcription suppressors, nucleoside inhibitors, can be one of the strategies which may have a better outcome. Therapeutic use of MSc-derived exosome and interferon as nanoparticles via aerosol is a promising approach. Host-dependent RNA editing of the SARS-CoV-2 genome and regulating the cytoplasmic innate immunity may be yet another approach to decease infectivity and severity of COVID -19.

REFERENCES

[1]   Reik W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature. 2007;447(7143):425-32.

[2]   Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes and Development. 2009;23(7):781-83.

[3]   Tiwari S. Recent advancement in methodology for understanding epigenetic modifications. J Clin Epigenet. 2017;3(S2):21.

[4]   Gräff J, Mansuy IM. Epigenetic dysregulation in cognitive disorders. Eur J Neurosci. 2009;30(1):01-08.

[5]   Cascella M, Rajnik M, Aleem A, Dulebohn S, Di Napoli R. Features, evaluation, and treatment of coronavirus (COVID-19). 2022. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. PMID: 32150360.

[6]   Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. The Lancet. 2020;395(10229):1054-62.

[7]   Chlamydas S, Papavassiliou AG, Piperi C. Epigenetic mechanisms regulating COVID-19 Infection. Epigenetics. 2021;16(3):263-70.

[8]   Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: Consider cytokine storm syndromes and immunosuppression. The Lancet. 2020;395(10229):1033-34.

[9]   Smale ST, Tarakhovsky A, Natoli G. Chromatin contributions to the regulation of innate immunity. Annual Review of Immunology. 2014;32:489-511.

[10] Cazaly E, Saad J, Wang W, Heckman C, Ollikainen M, Tang J. Making sense of the epigenome using data integration approaches. Frontiers in Pharmacology. 2019;10:126.

[11] Atlante S, Mongelli A, Barbi V, Martelli F, Farsetti A, Gaetano C. The epigenetic implication in coronavirus infection and therapy. Clinical Epigenetics. 2020;12(1):01-02.

[12] Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology. 2013;38(1):23-38.

[13] Menachery VD, Schäfer A, Burnum-Johnson KE, Mitchell HD, Eisfeld AJ, Walters KB, et al. MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic landscape. Proceedings of the National Academy of Sciences. 2018;115(5):E1012-21.

[14] Schäfer A, Baric RS. Epigenetic landscape during coronavirus infection. Pathogens. 2017;6(1):8.

[15] García-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: A lesson in detente. Science. 2006;312(5775):879-82.

[16] Van Lint C, Emiliani S, Ott M, Verdin E. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. The EMBO Journal. 1996;15(5):1112-20.

[17] Liang Y, Vogel JL, Narayanan A, Peng H, Kristie TM. Inhibition of the histone demethylase LSD1 blocks α-herpesvirus lytic replication and reactivation from latency. Nature Medicine. 2009;15(11):1312-17.

[18] Felsenfeld G, Groudine M. Controlling the double helix. Nature. 2003;421(6921):448-53.

[19] Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693-705.

[20] Lichinchi G, Gao S, Saletore Y, Gonzalez GM, Bansal V, Wang Y, et al. Dynamics of the human and viral m 6 A RNA methylomes during HIV-1 infection of T cells. Nature Microbiology. 2016;1(4):01-09.

[21] Kennedy EM, Bogerd HP, Kornepati AV, Kang D, Ghoshal D, Marshall JB, et al. Posttranscriptional m6A editing of HIV-1 mRNAs enhances viral gene expression. Cell host and microbe. 2016;19(5):675-85.

[22] Menachery VD, Eisfeld AJ, Schäfer A, Josset L, Sims AC, Proll S, et al. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. MBio. 2014;5(3):e01174-14.

[23]. Mueller AL, McNamara MS, Sinclair DA. Why does COVID-19 disproportionately affect older people? Aging (Albany NY). 2020;12(10):9959.

[24] Holt NR, Neumann JT, McNeil JJ, Cheng AC, Unit HE, Prahan V. Implications of COVID-19 in an ageing population. Med J Aust. 2020;382:2081-90.

[25] Vankadari N, Wilce JA. Emerging COVID-19 coronavirus: Glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerging microbes and infections. 2020;9(1):601-04.

[26] WHO (2020b). Solidarity clinical trial for COVID-19 treatments. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-COVID-19-treatments (Accessed on October 4, 2021).

[27] Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459-68.

[28] Zhang L, Liu Y. Potential interventions for novel coronavirus in China: A systematic review. Journal of Medical Virology. 2020;92(5):479-90.

[29] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Research. 2020;30(3):269-71.

[30] Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nature Reviews Drug Discovery. 2020;19(3):149-50.

[31] Bonam SR, Muller S, Bayry J, Klionsky DJ. Autophagy as an emerging target for COVID-19: Lessons from an old friend, chloroquine. Autophagy. 2020;16(12):2260-66.

[32] Sabine Hazan, ProgenaBiome, 'A Randomized, Double-Blind, Placebo-Controlled Phase IIa Study of Hydroxychloroquine, Vitamin C, Vitamin D, and Zinc for the Prevention of COVID-19 Infection', June 22, 2020, NCT number: NCT04335084. (Accessed on November 28th, 2021).

[33] Kreft H, Jetz W. Global patterns and determinants of vascular plant diversity. Proceedings of the National Academy of Sciences. 2007;104(14):5925-30.

[34] Saksena N, Bonam SR, Miranda-Saksena M. Epigenetic lens to visualize the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection in COVID-19 pandemic. Frontiers in Genetics. 2021;12:291.

[35] Worldwide Covid-19 Candidate Vaccines. Indian Council of Medical Research. (Accessed on 10th July, 2021). URL: https://vaccine.icmr.org.in/covid-19-vaccine.

[36] Darbar S, Agarwal S, Saha S. COVID19 Vaccine: COVAXIN®-India's first indigenous effective weapon to fight against Coronavirus (A Review). Parana Journal of Science and Education. 2021;7(3):01-09.

[37] Jones I, Roy P. Sputnik V COVID-19 vaccine candidate appears safe and effective. The Lancet. 2021;397(10275):642-43.

[38] Moderna COVID-19 Vaccine. Department of health & human service–USA. CDC. (Accessed on 10th July, 2021). URL: https://www.cdc.gov/vaccines/covid-19/info-by-product/moderna/downloads/storage-summary.pdf.

[39] COVID-19 mRNA Vaccine BNT162b2 (Pfizer). BC Centre for Disease Control. (Accessed on 10th July 2021). URL: http://www.bccdc.ca/resource-gallery/Documents/Guidelines%20and%20Forms/Guidelines%20and%20Manuals/Epid/CD%20Manual/Chapter%202%20-%20Imms/Part4/COVID-19_mRNA_Vaccine_BNT162b2_Pfizer-BioNTech.pdf.

[40] Livingston EH, Malani PN, Creech CB. The Johnson & Johnson Vaccine for COVID-19. JAMA. 2021;325(15):1575.

[41] Ghiasi NV, Arabsorkhi M, Hoseyni TS, Esfandiari K, Sadighpour T, Jahantigh HR. Efficacy and side effects of Sputnik V, Sinopharm and AstraZeneca vaccines to stop COVID-19; A review and discussion. Immunopathologia Persa. 2021;7(2):31.

[42] Nidhi Parekh (22 July 2020). "CoronaVac: A COVID-19 Vaccine Made From Inactivated SARS-CoV-2 Virus". Accessed on November 4, 2021.

[43] Minseo Jeong. Sinovac COVID-19 vaccine: What are the side effects?. Medical News Today. June 12, 2021. (Accessed on November 4, 2021). https://www.medicalnewstoday.com/articles/sinovac-covid-19-vaccine-what-are-the-side-effects.

[44] Sushmita Choudhury. All about India's, and world's, first DNA Covid vaccine. Times of India. Oct 4, 2021, TIMESOFINDIA.COM. (Accessed on November 4, 2021). https://timesofindia.indiatimes.com/india/all-about-indias-and-worlds-first-dna-covid-vaccine/articleshow/84140612.cms#:~:text=According%20to%20Zydus%20Cadila%2C%20its,challenges%20leading%20to%20vaccine%20wastage.

[45] DNA Web Team. All you need to know about 3 dose Zydus Cadila's ZyCoV-D vaccine to be administered to children. DNA India. Aug 21, 2021. (Accessed on November 4, 2021). https://www.dnaindia.com/india/photo-gallery-all-you-need-to-know-about-3-dose-zydus-cadila-s-zycov-d-vaccine-to-be-administered-to-children-2907082/zycov-d-vaccine-is-needle-free-2907083.

[46] Content Creation Staff (Fact checked by Robert Carlson). INO-4800 COVID-19 Vaccine. Precision Vaccinations. Updated November 10, 2021. (Accessed on November 4, 2021). https://www.precisionvaccinations.com/vaccines/ino-4800-covid-19-vaccine.

[47] Tebas P, Yang S, Boyer JD, Reuschel EL, Patel A, Christensen-Quick A, et al. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, Phase 1 clinical trial. Clin Med. 2021;31:100689.

[48] Wadman M (November 2020). "The long shot". Science. 370 (6517):649-653. Bibcode:2020Sci…370..649W. Doi: 10.1126/science.370.6517.649. PMID 33154120.

[49] Chung YH, Beiss V, Fiering SN, Steinmetz NF. "COVID-19 vaccine frontrunners and their nanotechnology design". ACS Nano. 2020;14(10):12522-37.

[50] Jonathan Corum and Carl Zimmers. How the Novavax Vaccine Works. The New York Times. Updated May 7, 2021. (Accesse d on November 4, 2021). https://www.nytimes.com/interactive/2020/health/novavax-covid-19-vaccine.html.

[51] Dr. Wimmer. Covi-VAC for SARS-CoV-2. Sep 4,2021. CODAGENIX.COM. (Accessed on November 15, 2021). https://codagenix.com/vaccine-programs/covid-19/.

[52] Maria Kiselyova. Russia's CoviVac more than 80% effective against COVID-19-Ifax. Healthcare & Pharmaceuticals. Reuters. June 2, 2021 www.reuters.com (Accessed on November 4, 2021). https://www.reuters.com/business/healthcare-pharmaceuticals/russias-covivac-more-than-80-effective-against-covid-19-ifax-2021-06-02/.

[53] de Araújo JT, Lima LA, Vale EP, Martin-Pastor M, Lima RA, de Barros Silva PG, et al. Toxicological and genotoxic evaluation of anacardic acid loaded-zein nanoparticles in mice. Toxicology Reports. 2020;7:1207-15.

[54] Dekker FJ, van den Bosch T, Martin NI. Small molecule inhibitors of histone acetyltransferases and deacetylases are potential drugs for inflammatory diseases. Drug Discovery Today. 2014;19(5):654-60.

[55] El Baba R, Herbein G. Management of epigenomic networks entailed in coronavirus infections and COVID-19. Clinical Epigenetics. 2020;12(1):01-02.

[56] van Dam PA, Huizing M, Mestach G, Dierckxsens S, Tjalma W, Trinh XB, et al. SARS-CoV-2 and cancer: Are they really partners in crime? Cancer Treat Rev. 2020;89:102068.

[57] Jit BP, Qazi S, Arya R, Srivastava A, Gupta N, Sharma A. An immune epigenetic insight to COVID-19 infection. Epigenomics. 2021;13(06):465-80.

[58] Johns Hopkins University. Decitabine for Coronavirus (COVID-19) Pneumonia-Acute Respiratory Distress Syndrome (ARDS) Treatment: DART Trial (DART). U.S. National Library of Medicine. ClinicalTrials.gov. July 22, 2020. (Accessed on October 4, 2021). https://clinicaltrials.gov/ct2/show/NCT04482621.

[59] Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, Curtis N, van Crevel R, van de Veerdonk FL, et al. Trained immunity: A tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. Cell. 2020;181(5):969-77.

[60] Kerboua KE. The perplexing question of trained immunity vs adaptive memory in COVID-19. Journal of Medical Virology. 2020;92(10):1858-63.

[61] Geller A, Yan J. Could the induction of trained immunity by β-glucan serve as a defense against COVID-19? Frontiers in Immunology. 2020;11:1782.

[62] Singh V. Can vitamins, as epigenetic modifiers, enhance immunity in COVID-19 patients with non-communicable disease? Curr Nutr Rep. 2020:01-08.

[63] Vyas N, Kurian SJ, Bagchi D, Manu MK, Saravu K, Unnikrishnan MK, et al. Vitamin D in prevention and treatment of COVID-19: Current perspective and future prospects. J Am Coll Nutr. 2021;40(7):632-45.

[64] Fang Y, Yang C, Yu Z, Li X, Mu Q, Liao G, et al. Natural products as LSD1 inhibitors for cancer therapy. Acta Pharmaceutica Sinica B. 2021;11(3):621-31.

[65] Pruimboom L. Methylation pathways and SARS-CoV-2 lung infiltration and cell membrane-virus fusion are both subject to epigenetics. Frontiers in Cellular and Infection Microbiology. 2020;10:290.

[66] Ayaz S, Crea F. Targeting SARS-CoV-2 using polycomb inhibitors as antiviral agents. Epigenomics. 2020;12(10):811-12.

[67] Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. BMC Biology. 2019;17(1):01-20.

[68] Shirvaliloo M. Epigenomics in COVID-19; the link between DNA methylation, histone modifications and SARS-CoV-2 infection. Epigenomics. 2021;13(10):745-50.

[69] Clem AS. Fundamentals of vaccine immunology. Journal of Global Infectious Diseases. 2011;3(1):73.

[70] Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature. 2001;410(6832):1099-103.

[71] Golonka RM, Saha P, Yeoh BS, Chattopadhyay S, Gewirtz AT, Joe B, et al. Harnessing innate immunity to eliminate SARS-CoV-2 and ameliorate COVID-19 disease. Physiological Genomics. 2020;52(5):217-21.

[72] Lu M, Zhang Z, Xue M, Zhao BS, Harder O, Li A, et al. N6-methyladenosine modification enables viral RNA to escape recognition by RNA sensor RIG-I. Nat Microbiol. 2020;5(4):584-98.

[73] Zhao Y, Lukiw WJ. Microbiome-mediated upregulation of microRNA-146a in sporadic Alzheimer's disease. Front Neurol. 2018;9:145.

[74] Quigley EMM, Gajula P. Recent advances in modulating the microbiome. F1000Res. 2020 Jan 27;9:F1000 Faculty Rev-46.

[75] To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study. The Lancet Infectious Diseases. 2020;20(5):565-74.

[76] Yamamoto S, Saito M, Tamura A, Prawisuda D, Mizutani T, Yotsuyanagi H. The human microbiome and COVID-19: A systematic review. PloS one. 2021;16(6):e0253293.

[77] Lopez-Santamarina A, Lamas A, Del Carmen Mondragón A, Cardelle-Cobas A, Regal P, Rodriguez-Avila JA, et al. Probiotic Effects against Virus Infections: New Weapons for an Old War. Foods. 2021;10(1):130.

[78] Kanauchi O, Andoh A, AbuBakar S, Yamamoto N. Probiotics and paraprobiotics in viral infection: Clinical application and effects on the innate and acquired immune systems. Current pharmaceutical design. 2018;24(6):710-77.

[79] Harata G, He F, Hiruta N, Kawase M, Kubota A, Hiramatsu M, et al. Intranasal administration of *Lactobacillus rhamnosus* GG protects mice from H1N1 influenza virus infection by regulating respiratory immune responses. Letters in Applied Microbiology. 2010;50(6):597-602.

[80] Channappanavar R, Perlman S. Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39(5):529-39.

[81] Hossein-Khannazer N, Shokoohian B, Shpichka A, Aghdaei HA, Timashev P, Vosough M. An update to "novel therapeutic approaches for treatment of COVID-19". Journal of Molecular Medicine. 2021;99(2):303-10.

[82] Di Giorgio S, Martignano F, Torcia MG, Mattiuz G, Conticello SG. Evidence for RNA editing in the transcriptome of 2019 Novel Coronavirus. bioRxiv. 2020 Jan 1.

[83] Tomaselli S, Galeano F, Locatelli F, Gallo A. ADARs and the balance game between virus infection and innate immune cell response. Current Issues in Molecular Biology. 2015;17(1):37-52.

[84] Sa Ribero M, Jouvenet N, Dreux M, Nisole S. Interplay between SARS-CoV-2 and the type I interferon response. PLoS Pathogens. 2020;16(7):e1008737.

[85] Pujantell M, Riveira-Muñoz E, Badia R, Castellví M, Garcia-Vidal E, Sirera G, et al. RNA editing by ADAR1 regulates innate and antiviral immune functions in primary macrophages. Scientific Reports. 2017;7(1):01-04.

[86] Di Giorgio S, Martignano F, Torcia MG, Mattiuz G, Conticello SG. Evidence for host-dependent RNA editing in the transcriptome of SARS-CoV-2. Science Advances. 2020;6(25):eabb5813.

[87] Nehme Z, Pasquereau S, Herbein G. Control of viral infections by epigenetic-targeted therapy. Clinical Epigenetics. 2019;11(1):01-07.

PARTICULARS OF CONTRIBUTORS:
1. Postgraduate, Department of Biotechnology, Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India.
2. Postgraduate, Department of Biotechnology, Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Mr. Rahul Saxena,
SRHU, HIHT, Jolly Grant, Dehradun, Uttarakhand, India.
E-mail: rahulsaxena.1701@gmail.com

AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was informed consent obtained from the subjects involved in the study?   NA
• For any images presented appropriate consent has been obtained from the subjects.   NA