Review

Probing the Immune System Dynamics of the COVID-19 Disease for Vaccine Designing and Drug Repurposing Using Bioinformatics Tools

Deepshikha Yadav 1,2, Shriya Agarwal 3, Pranav Pancham 4, Divya Jindal 4, Vinayak Agarwal 4, Premshankar Kumar Dubey 1,2, Saurabh K. Jha 5,*, Shalini Mani 4, Rachana 4, Abhijit Dey 6, Niraj Kumar Jha 5,*, Kavindra Kumar Kesari 7,*, and Manisha Singh 4,*

1 Physico-Mechanical Metrology Division, CSIR—National Physical Laboratory, New Delhi 110012, India; deepshikha.yadav93@gmail.com (D.Y.); premkdubey@gmail.com (P.K.D.)
2 Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India
3 Department of Molecular Sciences, Macquarie University, Sydney, NSW 2109, Australia; shriyaz.dd@gmail.com
4 Department of Biotechnology, Jaypee Institute of Information Technology (JIIT), Noida 201309, India; pranavpanchm@gmail.com (P.P.); divyajindal065@gmail.com (D.J.); vinayakagarwal37@gmail.com (V.A.); mani.shalini@gmail.com (S.M.); rachana.dr@iitbombay.org (R.)
5 Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida 201310, India; nirajkumarjha2011@gmail.com
6 Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata 700073, India; abhijit.dbs@presiuniv.ac.in
7 Department of Applied Physics, School of Science, Aalto University, 02150 Espoo, Finland
* Correspondence: saurabh.jha@sharda.ac.in (S.K.J.); kavindra.kesari@aalto.fi (K.K.K.); manishasingh1295@gmail.com (M.S.)

Abstract: The pathogenesis of COVID-19 is complicated by immune dysfunction. The impact of immune-based therapy in COVID-19 patients has been well documented, with some notable studies on the use of anti-cytokine medicines. However, the complexity of disease phenotypes, patient heterogeneity and the varying quality of evidence from immunotherapy studies provide problems in clinical decision-making. This review seeks to aid therapeutic decision-making by giving an overview of the immunological responses against COVID-19 disease that may contribute to the severity of the disease. We have extensively discussed theranostic methods for COVID-19 detection. With advancements in technology, bioinformatics has taken studies to a higher level. The paper also discusses the application of bioinformatics and machine learning tools for the diagnosis, vaccine design and drug repurposing against SARS-CoV-2.

Keywords: immunotherapy; coronavirus; viral mutations; interferons; epitope designing; vaccine designing; drug repurposing

1. Introduction

The history of the novel coronavirus goes back to the nineteenth century when it was infecting cats and causing high fever and a swollen belly, making it the first-ever reported case of infection by a coronavirus. At that time, it was infecting other animals too such as pigs and chickens, but the veterinary doctors were unaware. It was only after the discovery of two viruses in the UK and US, possessing crown-like structures causing the common cold in humans, that established the relationship between the viruses infecting both humans and animals, which had similar structures. These viruses were then studied under electron microscopes and it was concluded that they resemble the “solar corona”. The term “coronaviruses” was coined in 1968. The term was derived from the Greek word корона, meaning crown for the entire group. However, in our century, researchers have encountered highly pathogenic CoVs such as SARS-CoV and MERS-CoV, causing outbreaks.
that had originally been initiated in China in 2003 [1] and Saudi Arabia in 2012, respectively. The outbreak soon spread to other countries causing horrible morbidity and mortality. COVID-19 is the third CoV outbreak recorded in the history of human beings. This novel strain of coronavirus (SAR-CoV-2) was first detected in Wuhan in 2019, a city in the Hubei province of China, and has now spread to around 200 countries. This outbreak was labelled as a global pandemic by the WHO in 2019. Four coronavirus genera (α, β, γ, δ) have been identified so far, with human coronaviruses (HCoVs) detected in the α coronavirus (HCoV-229E and NL63) and β coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera [2–6]. The coronavirus is now becoming dangerous with time. Mortality due to the virus is at its highest peak, which has led the scientific community to gather as much information as possible on the biology of the virus.

The coronavirus family is a family of deadly pathogenic viruses and has persisted for the past 300 million years with the identification of dozens of coronavirus strains so far as mentioned in Figure 1. However, only seven strains are known to infect humans and four strains are known to cause the common cold. The four strains have originated from rodents (OC43 and HKU1) and bats (229E and NL63). Most recently, it has been reported that in China that more than 500 CoVs have been identified in bats [7,8]. The three most dangerous strains, namely SARS-CoV, MERS-CoV, and SAR-CoV-2, are giving rise to severe associated diseases inflicting damage to the respiratory tract, namely severe acute respiratory syndrome (SARS), the Middle East respiratory syndrome (MERS), and COVID-19, respectively. SARS-CoV-2 has originated from bats owing to around 80% sequence homology between SAR-CoV-2 and SAR-CoV found in bats. Further, the structural analysis reports state that this RNA virus is much larger than its family members with a size of around 125 nm, and possesses the largest genomes (30 Kb in size) having 32–43% GC content [5,9–11]. The family of coronaviruses consists of enveloped viruses consisting of nucleoprotein (a positive-sense single-stranded RNA) encased within a capsid which is made up of matrix protein. The envelope consists of club-shaped glycoproteins projections. They possess several small open reading frames (ORFs) intertwined between conserved genes (ORF1ab, spike, envelope, membrane and nucleocapsid) located downstream of the nucleocapsid gene across different corona lineages. The coronavirus family has been classified into three groups based on their antigenic similarities. The first two groups constitute the mammalian coronaviruses, whereas the third group constitutes the avian coronaviruses [12–14]. Now in the current scenario, the world has witnessed the fastest technical assistance and alternative, provided by bioinformatics (BI) tools and programs in not only identifying the characteristics and probable existence of the deadly coronavirus but also playing an essential role in directing the theranostics approaches against COVID-19 infections. Subsequently, the CADD (Computer-Aided Drug Design) approach has shown remarkable development in the drug repurposing of various existing drugs in the market as well as identifying new antiviral compounds. Additionally, BI tools immensely helped in locating the viral genome data, prediction for protein–peptide, protein–drug, protein–protein docking, identifying the antigenic epitopes and antibody structures along with vaccine development, etc. In this review, we have tried to summarize the utility and efficacy of BI tools in developing various theranostics approaches to combat the COVID-19 situation.
pressure of the host immune system. This deletion coincides with the one found in the omicron (BA.1) variant, which was first identified in Botswana and South Africa in late November 2021. Another few more variants are being thoroughly examined, namely the P2 variant in Brazil and the CAL.20C variant in California [22–24]. At the end of November 2021, the omicron variant of coronavirus was detected with more than 32 mutations in the spike protein [25]. Most recently, another recombinant strain has been identified in the UK which is a recombinant of the omicron/BA.1 and its subvariant BA.2. According to the WHO, it has been named the most transmissible variant so far [26]. The regions of the mutations found in the different variants of SARS-CoV-2 are listed in the Table 1 below.

2. Viral Mutations

Replication of the virus leads to ample mutations in the SARS-CoV-2 genome. A sampling of the genomes of the SARS-CoV-2 virion particles mostly yielded mutations that are neutral or mildly deleterious. However, viral mutations that might confer fitness to the virus occur and may affect the pathogenicity, infectivity, transmissibility and/or antigenicity of the virus [15]. The virus mutated at a rate of approximately two mutations/month from December 2019 to October 2020 [16,17]. Even though the viral outbreak has been catastrophic, the virus has adopted a milder phenotype from the end of May 2020 [18]. Mutation in the ORF-8 has been shown to have resulted in a less severe viral strain, this region is involved in immune evasion and deletion of this region led to a robust immune response against the variant. This deletion might have been selected as a result of the pressure of the host immune system. This deletion coincides with the one found in the ORF-8 of the SARS-CoV-1 virus [19]. According to the WHO, mutations in the viral genome that affect the efficiency of the host immune system have occurred in several countries worldwide by the end of 2020. The mutations may enhance their ability to evade immune surveillance [20] by antibodies, thereby evading the humoral immune response of the body. These include the B.1.1.7 (α-variant), B.1.351 (β-variant) and B1.1.28 (E484K variant) from the UK, the B.1.351/501.YV2 variant from South Africa, the B.1.1.248/B1.1.28/P1 variant from Brazil and the Cluster 5 variant found in Denmark and Netherlands. Another variant, omicron (BA.1), was first identified in Botswana and South Africa in late November 2021 [21]. The B.1.351 variant has a mutated receptor-binding domain (RBD), protecting the virus against neutralizing antibodies. This mutation leads to higher transmission rates due to the improved affinity of the RBD towards the ACE-2 receptor. Although no variant has yet been found to confer vaccine resistance, the virus will continue to accumulate more variations under the pressure of vaccination. In addition to the variants already discussed, a few more variants are being thoroughly examined, namely the P2 variant in Brazil and the CAL.20C variant in California [22–24]. At the end of November 2021, the omicron variant of coronavirus was detected with more than 32 mutations in the spike protein [25]. Most recently, another recombinant strain has been identified in the UK which is a recombinant of the omicron/BA.1 and its subvariant BA.2. According to the WHO, it has been named the most transmissible variant so far [26]. The regions of the mutations found in the different variants of SARS-CoV-2 are listed in the Table 1 below.
Table 1. Regions of mutations in the different SARS-CoV-2 variants.

| Type of Variant | Region of Viral Mutations | Types of Mutations | References |
|-----------------|---------------------------|--------------------|------------|
| α-variant       | Spike protein             | 60–70 del, 145 del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H | [27]       |
| β-variant       | Spike protein             | K417N, E484K and N501Y | [28]       |
| Epsilon variant | Spike protein             | S13I, W152C and L452R | [29]       |
| γ-variant       | Spike protein             | K417T, E484K, N501Y  | [30]       |
| ηα-variant      | Spike protein             | E484K              |            |
| Cluster 5       | Spike protein             | ΔH69/ΔV70 deletion  | [32]       |

3. Coronavirus Adaptations

The killer viral strain SARS-CoV-2 has emerged as a powerful pathogen due to its robust adaptations enabling it to survive for longer periods. The novel coronavirus has been categorized as one of the most contagious diseases. Since the inception of COVID-19, it has been an incredibly difficult journey to come up with a vaccine against the virus. Unlike other viruses with high mutation rates, it is known to possess a competent proofreading system allowing it to attack the host cells at multiple sites and amongst them, the respiratory system is the primary target [33]. It is a widely known fact that most viruses lack a proofreading mechanism which ultimately limits their ability to protect their genome against mutations. However, the genome of the coronavirus is extremely stable due to the presence of a sophisticated proofreading system [33]. The virus adopts a unique process that may protect against vaccines. They evolve through the process of recombination by swapping sequences of RNA with another coronavirus. When two distant coronavirus types end up in the same cell, they undergo recombination. This recombination generates a mutated viral strain that possesses the ability to infect new cell types and transverse to other species [34,35]. One of the favourable organisms that house as many as 61 coronavirus species are the bats and, therefore, these viruses undergo recombination inside the bats without infecting them [36,37].

4. Diagnosis of Coronavirus

Patients infected with the coronavirus suffer from pneumonia-like symptoms, including fever, shortness of breath, sputum production and myalgia or fatigue. The virus majorly infects the upper respiratory tract (URTIs), along with infecting other tissues such as the digestive tract (diarrhoea, poor appetite, nausea and vomiting), nervous system (confusion and headache) and cardiovascular system (palm’s, chest distress and cardiac injury) in the body [38]. The different techniques used in the diagnosis of SARS-CoV-2 are described below:

- Nucleic acid amplification test (NAAT): The most reliable and accurate methodology for the detection of SARS-CoV-2 is the nucleic acid amplification test (NAAT) [39,40]. The most popular laboratory-based NAAT is the quantitative reverse transcription-polymerase chain reaction [41,42]. The samples that are used for diagnosis include sputum, saliva, nasal, pharyngeal and tracheal swabs, broncho-alveolar lavage, pleural effusion fluid, blood, faeces and sometimes urine and semen [6]. According to the World Health Organization (WHO), the detection of a single RNA sequence of coronavirus by RT-PCR is enough for the confirmation of the disease. This test is used for the real-time qualitative detection of nucleic acids from suspected viral pathogens [43]. In case of detection of SARC-CoV-2, the nucleic acid (RNA) isolated from the specific sample is first reverse transcribed into cDNA and then amplified. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle [43]. According to the WHO, based on the first sequences of SARS-CoV-2 made available on the GISAID database on 11 January
2020, primers and probes (nCoV_IP2 and nCoV_IP4) were designed to target the RdRp gene spanning nt 12,621–12,727 and 14,010–14,116 [44]. The current test kits may lead to false-negative results because the detection of COVID-19 in the early stages of the infection is challenging due to the improper isolation of RNA or inadequate methods for detection.

- Chest computerized tomography (CT): CT scans are used for the diagnosis and imaging of viral pneumonia. It has been extensively used for the timely diagnosis and treatment of other coronavirus outbreaks caused by SARS-CoV and MERS-CoV. It is a confirmatory scan that is used to detect any false negatives as robust detection of COVID-19 is imperative for avoiding these false negatives because a CT scan can detect the infection even before the manifestation of symptoms resulting in timely treatment [39].

- Serological immunoassays: A plethora of serological immunoassays exists which detect SARS-CoV-2 viral proteins and antibodies against those proteins in the plasma or serum. The most popular biomolecules detected by commercial immunological tests such as rapid lateral flow immunoassay (LFIA) tests, automated chemiluminescence immunoassay (CLIA) and manual ELISA and other formats are IgM and IgG antibodies. The antibodies are released into the bloodstream in the second week of viral infection. IgM and IgG may be detected within 10–30 days and 20 days post-infection, respectively [40]. The IgM antibody unveils a drop in concentration, whereas IgG persists in the systemic circulation for prolonged periods and may play a role in adaptive immunity against SARS-CoV-2 in a possible second encounter. ELISA kits against nucleocapsid (NP) and spike (SP) viral proteins exist in the market but they are mainly used for research and development purposes only [45]. The details of all the diagnostic techniques used for SARS-CoV-2 detection are summarized in the Table 2 below:

| S. No. | Diagnostic Method | Principle of Method | Sample | Time Duration | Detected Component | References |
|-------|------------------|---------------------|--------|---------------|-------------------|------------|
| 1.    | RT-PCR           | Polymerase chain reaction | Sputum, saliva, nasal, phalangeal and tracheal swabs, broncho-alveolar lavage, pleural effusion fluid, blood, faeces, urine and semen | 6–8 h | Viral RNA | [6,46,47] |
| 2.    | PCR with fluorescently labelled probes | Polymerase chain reaction | RNA | 6–8 h | Viral RNA | [41] |
| 3.    | Chest computerized tomography (CT) | X-ray | Chest X-ray | 30–60 min | Small nodules in the chest | [48–50] |
| 4.    | Rapid lateral Flow Immunoassay (LFIA) | A liquid sample consisting of analyte transports without the help of capillary action through 3 zones of polymeric strips, upon which molecules that can interact with the analyte are attached | Throat swab or sputum | IgG | [51] |

Table 2. Diagnostic tests for SARS-CoV-2 detection.
Table 2. Cont.

| S. No. | Diagnostic Method                                      | Principle of Method                                                                 | Sample                        | Time Duration | Detected Component                                                      | References |
|--------|--------------------------------------------------------|------------------------------------------------------------------------------------|-------------------------------|---------------|----------------------------------------------------------------------|------------|
| 5.     | Automated chemiluminescence immunoassay (CLIA)         | Chemiluminescent methods utilizing luminophore markers                            | Serum                         |               | IgM and IgG antibodies                                                 | [52]       |
| 6.     | Manual ELISA                                           | Specific antibody-antigen interactions                                             | Saliva, serum, plasma          | 5–6 h         | IgG antibodies                                                         | [53,54]    |
| 7.     | Rapid antigen test                                     | Rapid membrane-based lateral flow immunoassay                                     | Saliva                        | 15–30 min     | nucleocapsid protein antigen of the coronavirus SARS-CoV-2           | [55]       |
| 8.     | Detection of D-dimer levels                            | Coagulation                                                                        | Blood                         | 1–2 days      | D-dimer concentrations                                                 | [56]       |
| 9.     | Microbial culture test                                 | NAAT (nucleic acid amplification test)                                             | Nucleic acids                 | 2–3 days      | Viral growth                                                           | [57]       |
| 10.    | Lateral flow antigen test                              | Immunochromatographic assay                                                       | Throat swab or sputum          | 30–60 min     | Nucleocapsid protein antigen                                           | [58]       |
| 11.    | Neutralizing antibody test                             | Rapid membrane-based lateral flow immunoassay                                     | Serum, throat swab or sputum   | 30–60 min     | Neutralizing antibodies                                                | [59]       |

4.1. Molecular Targets for Diagnostic Kits

The disease-specific biomarkers may serve as effective diagnostic tools. As a result, in vitro diagnostic kits are being designed for the early detection of SARS-CoV-2. The most common receptor through which SARS-CoV-2 enters the host system is the angiotensin-converting enzyme receptor (ACE-2) which is abundantly found in the lower respiratory tract [48–50]. SP glycoprotein is a viral ligand that binds to the ACE-2 receptor through its receptor-binding domain which is found in one of the two subunits (S1) of the SP protein (S1 and S2). S2, on the other hand, aids in membrane fusion [60,61]. This SP protein serves as a target of various neutralizing antibodies and vaccines. NP, a phosphor protein, is another important viral ligand that is produced and divided into copious amounts during viral infection [62]. However, both the viral ligands, the SP and NP glycoproteins, possess high immunogenicity. The highest amount of NP is detected after 10 days of viral infection which can be easily detected using a sandwich immunoassay [63]. The most abundant membrane protein (MP) and the smallest structural envelope protein (EP), which are involved in important viral functions such as assembly, release and pathogenesis, may also serve as potential targets for viral detection kits [37,64–66].

D-Dimer-Based Detection

D-dimer detection is used for fast diagnosis of COVID-19 infection, as abnormal coagulation is closely associated with its progression. The severity of the infection can be determined by D-dimer levels, which are protein fragments formed when there is clotting of blood. Higher levels of D-dimer showed a higher probability of pulmonary embolism in patients with COVID-19 infection [67].

The advantages and disadvantages of the different tests are described in the Table 3 below:
Table 3. Advantages and disadvantages of various diagnostic tests used for SARS-CoV-2 detection.

| Diagnostic Technique | Advantages                                                                 | Disadvantages                                                                 | References |
|----------------------|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------|
| NAAT                 | • High specificity <br> • High sensitivity <br> • High accuracy <br> • Ability to bind to multiple targets enabling the detection of mutated viral strains | • Low sensitivity during early infection <br> • Contamination issues <br> • Time-consuming sample handling <br> • Expensive equipment, reagents <br> • Requirement of trained personnel <br> • Variable detection of SARS-CoV-2 depending on the type of clinical specimen <br> • False-negative results depending on the time of sample collection or the improper isolation of genetic material <br> • Changes in diagnostic accuracy throughout the disease <br> • Long turnaround times that fail to prevent transmission | [68,69] |
| CT scan              | • Early detection of infection <br> • High accuracy                          | • Multiple bilateral ground-glass opacities in the peripheral lower lung zones are also seen in patients with SARS-CoV and MERS-CoV infections <br> • False positives, because of other causes of pneumonia-like seasonal flu <br> • Normal CT scan results in certain COVID-19 patients <br> • The contagious nature of the disease disposes healthcare providers and other patients at risk of infection | [70,71] |
| Serological immunoassays | • Low cost <br> • Rapid detection times <br> • Rapid testing enables the control of outbreaks | • Lack of specificity leading to false positives because of the presence of cross-reactivity with other coronaviruses <br> • Low accuracy <br> • Lack of rigorous evaluation of the technique | [72] |

4.2. Effect of Viral Mutations on the Accuracy of Diagnostic Tests

Since the virus accumulates mutations over time, the analysis of the sequenced data from time to time is critical to evaluate their effect on the diagnostic tests [73]. If tests continuously give false-negative results, the viral genome must be immediately sequenced to fish out any mutations that may be responsible for the same. In addition, the popular NAAT is designed in a way that enables binding to multiple targets. Therefore, even if a mutation occurs concerning one target site, the test will continue to work [74]. For example, the widely known S-gene drop out/S-gene target failure of the Thermo Fisher TaqPath test may lead to false-negative test results as a consequence of the $\Delta 69/70$ mutation in the Spike-gene. However, the test continues to deliver accurate test results because of the presence of primers specific to two other target genes. Even though viral evolution is inevitable, the provision of multiple target binding in most diagnostic tests works as a boon in the fight against the pandemic. In addition, the S-gene failure occurred in the detection of the B.1.1.7 variant, and a majority of the PCR-based diagnostic tests do not target the S-gene, and even if they do, they work against multiple targets. S-gene failure is widely being used to screen variants from positive PCR results, if the S-gene failure occurs, the genome is sequenced to identify the potential mutations in the S-gene. However, the S-gene is not the only mutation that affects the diagnostic tests, and instances of target failure have been reported as a result of mutations in other genes [75]. Therefore, sequencing is an important tool to curb the problem of diagnostic test failure.
5. Transmission of Coronavirus

The transmission of this deadly virus occurs through the air when an infected person sneezes or coughs, or even talks. The microdroplets produced in the respiratory tract travel through the air and come in contact with the other person(s), which have a shelf life of approximately half an hour. The droplets are laced with virions and may enter the host, ultimately eliciting an infection [76]. Even though the virus can persist in aerosols for an only half-hour, it still settles onto surfaces later and can persist for longer time durations, up to 72 h depending upon the type of surface it comes in contact with, such as steel, cardboard, plastic, etc. [76]. About 80% of COVID-19 cases are asymptomatic (mild–moderate symptoms), 15% of cases progress to pneumonia and only about 5% of cases result in acute respiratory distress syndrome (ARDS), septic shock and/or multiple organ failure. The major cell surface receptor for the coronavirus through which it enters the host cell is the ACE-2 receptor. The ACE-2 receptor is scattered throughout the human body but is present abundantly in the epithelia of the lung and small intestine, serving as routes for viral entry [77].

6. Mechanism of Viral Entry into the Host

The major cell surface receptor for the coronavirus through which it enters the host cell is the ACE-2 receptor. The coronavirus is competent in taking over the host machinery by binding onto the ACE-2 receptor. The receptor viral complex is endocytosed inside the cell where the viral genetic material is released inside the host cell and replicates. The RNA is then translated into the essential viral proteins which are assembled to form viral particles and released from the Golgi apparatus in the form of vesicles. Once released the viral particles are exported out of the cell and eventually start infecting others in the same order. The mechanism of viral entry in the host system is depicted in Figure 2.

![Figure 2. Diagram representing the mechanism by which coronavirus infiltrates the human system followed by its replication and spread.](image)

7. Immunogenic Trigger in Response to Coronavirus

Immune cells are constantly surveying the body to check for any foreign antigen that might have entered the system. The virus is pathogenic and can evade immune surveillance...
by residing inside the cell. Once it enters, the virus hijacks the host machinery and undergoes replication, translation and assembly [78]. The immune system is a highly evolved and complex system. Various mechanisms work together to eliminate these pathogens from the system. One way to get rid of the virus is through major histocompatibility complex I (MHC) proteins. The MHC-I molecules present viral proteins to a specialized class of cells known as “Cytotoxic T-cells” (T_c cells). T_c cells bear a receptor called a T-cell receptor (TCR) which can recognize peptide antigens bound to MHC class I proteins. The receptor then informs the T-cell of possible infection which kill the infected cell by releasing different killer molecules [79].

These killer molecules include perforins, granzymes, granulysin and cytokines such as interferons and tumour necrosis factors (TNFs) [80]. Interferons are particularly effective against viruses as they induce antiviral properties and act as signalling molecules by informing the neighbouring cells of a possible viral infection [81,82]. The coronavirus infection triggers the innate and adaptive immune response. Even though a rapid and well-organized immune response is necessary to get rid of the virus [83,84], an extreme inflammatory response and a poorly regulated adaptive immune response do more harm than good. They inflict on-site (viral entry) and systemic tissue injury [85].

7.1. Hallmarks of the Immune Response to COVID-19

The pro-inflammatory response leads to acute lung infection and ARDS which are the major factors responsible for the high mortality rate due to coronavirus infection. The hallmark event of the immune response against COVID-19 is a result of cytokines triggered by the pro-inflammatory molecules. A plethora of cytokines (IFN-a, IFN-g, IL-1b, IL-6, IL-12, IL-18, IL-33, TNF-a, TGFb, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) are released due to this response. This immune dysregulation plays an important role in defining the harmful after-effects of the corona infection that may cause death. Therefore, understanding the exact immune mechanism is essential for preventing the disease progress from a mild to a severe stage [84,86,87].

7.2. The Interplay between the Production of Interferons and Inflammatory Cytokines

Infection by COVID-19 involves the cells of the upper respiratory tract, majorly the cells of the bronchial epithelium and pneumocytes [88]. The virus enters the pneumocytes of the alveolar epithelium by binding to the ACE 2 receptor [89]. Immediately after entering the host, the immune system responds and down-regulates the expression of ACE-2 receptors by inducing autophagy and detaching the basal membrane. In addition to this, the immune system triggers the release of interferons. As a combat strategy, virions are produced in large amounts, resulting in the spread of the infection to neighbouring cells and the systemic circulation as ACE-2 receptors are expressed by many tissue types [90–93].

Viral pathogenesis is characterized by two major events, one is the drop in the production of interferons, mainly IFN-I and IFN-III, and the overexpression of different inflammatory cytokines, such as IL-1B, IL-6, TNF and IL1RA [87]. SARS-CoV-2 is unique in evading the immune system by down-regulating the production of interferons which are the most important antiviral mediators, and it also possesses the ability to replicate within the pulmonary tissue, traits that other coronaviruses do not possess [87,94].

Interleukins are released in abundant amounts and greatly impact the inflammation process and the severity of COVID-19 symptoms is governed by the release of interleukins, (IL-1beta and IL-8) and TNF-alpha [87,95].

A study by Carty et al. [96] showed the contribution of the IL-1 pathway in the pathogenesis of COVID-19. The cytokines such as IL2, IL6, TNF, IFNA1/13, IL1A, IL1B and the receptor IL1 were overexpressed only in infected patients in comparison to healthy individuals [97].

Apart from this, the innate immune system consists of a specialized recognition receptor known as toll-like receptors expressed by a variety of cell types including dendritic cells (DC), macrophages, lymphocytes and parenchymal cells which are involved in the
recognition of specific patterns found on the surface of pathogens [98]. Different types of TLRs bind to specific viral products which may include single-stranded DNA (TLR3 and 8) or double-stranded DNA (TLR9). In addition to TLRs, DExD/H box and RNA helicases are also involved in identifying viral pathogens. They are known to stimulate the expression of type-I interferons (IFN) and a variety of IFN-stimulated genes and inflammatory cytokines [99]. Innate immunity against viral infections by antiviral inflammatory responses, blockage of infection, protection of cells against infection and destruction or inhibition of virus infected cells is depicted in Figure 3.

**Figure 3.** Schematic representation of immunity against viral pathogens.

**Mechanism of Action of Interferons**

Similarly, it has also been reported that the innate immune response to the viral attack is mediated by specialized proteins known as interferons that bind to type-II IFN receptors and result in an antiviral state. They stimulate the expression of more than 100 IFN-stimulated genes, and the subsequent events drive the phenomenon of the antiviral state which is responsible for the inhibition of protein synthesis in the infected cell. This causes the halting of viral replication as the virus multiplies by hacking the protein synthesis machinery of the host cell. In addition to this, the type-I IFNs also activate the natural killer cells (NK) and produce cytokines that eventually trigger the NK-cell mediated antiviral responses [100].

NK cells are an important class of specialized cells that are involved in the production of pro-inflammatory cytokines. They kill infected cells and interact with dendritic cells, thus acting as an important component of the innate immune response against viral infections [101].

**7.3. Role of the Inflammasome in Causing Inflammation**

Infection by SARS-CoV-2 leads to the activation of cytoplasmic NLRP3 inflammasome (Table 1). Inflammasome activation in macrophages, epithelial cells and sometimes endothelial cells release pro-inflammatory cytokines, interleukin (IL)-1β and IL-18, which contribute to the pathogenic inflammation responsible for the severity of COVID-19 symptoms [102,103]. In addition, sensing viral RNA by toll-like receptors (TLR)3, TLR7, TLR8 and TLR9 activates the NF-κB signalling pathway and several pro-inflammatory cytokines with a major role in initiating virus-induced inflammation [104,105].
7.3.1. Histopathological Markers of Inflammation

Histopathological observations of pulmonary lesions in SARS cases not only show nonspecific inflammatory responses such as oedema and inflammatory cell infiltration but also exhibit severe ex-foliation of alveolar epithelial cells, alveolar septal widening, damage to alveolar septa and alveolar space infiltration in a distinctly organized manner. SARS-CoV-2 infection can cause pathological changes, such as degeneration, infiltration and hyperplasia. Damage to the pulmonary interstitial arteriolar walls indicates that inflammatory response plays an important role throughout the disease. These findings suggest that the body’s innate ability to respond to infection plays an essential role in the disease inflammation along with viral pathogenesis [106,107].

7.3.2. Role of Damage-Associated Molecular Patterns (DAMPs) Inflammation Trigger

Another molecule that is responsible for causing inflammation in the upper respiratory tract after COVID-19 infection is DAMP. It is released only under stressful conditions and induces inflammation by its binding to receptor P2X/P2Y. In addition, DAMP also acts via activation of the NLRP3 inflammasome, leading to the activation of caspase-1, for the production of IL-1 which is a potent pro-inflammatory cytokine [108–110].

7.4. Role of the Adaptive Immune System against COVID-19

Severe symptoms are due to an imbalanced immune response. The adaptive immune system targets the pathogenic virus by releasing antibodies specific to the infection and cytotoxic T-cells which defend against foreign invaders. A study by Clay et al. [90,111] showed that in a SARS-CoV primate model of infection, even though the virus replicates in the lungs for 10 days ensuing infection, inflammation was more evident post-viral clearance with a peak at day 14 post-infection until day 28. These findings indicate that the early phase is dependent on the viral presence, but a viral independent phase occurs at a much later stage post-viral clearance which is characterized by inflammation. This viral independent stage occurs because of epitope scattering, which is a result of prolonged tissue destruction by the virus. Studies need to be conducted to show if a similar two-phased progression occurs in the case of COVID-19 as well. The viral particles are attacked by B- and T-lymphocytes of the adaptive immune system. There is a dynamic interaction occurring at the cellular level, where the viral peptides are presented by the MHC class 2 molecules to CD8+ T-cells which significantly impact viral clearance by acting as killers. This encounter via the MHC molecules of the viral particle induces the activation of CD8+ cells, leading to their division and clonal expansion into virus-specific effector and memory T-cells. The viral particles are presented to CD4+ T-cells in a similar mechanism through the MHC class 1 molecule. The B-cells can identify viral antigens and interact with CD4+ T-cells [112].

The IgM antibody is released within a week of viral infection, followed by IgG antibody release which translates into lifelong immunity against the virus. Structural proteins of SARS-CoV-2 share genetic similarities with SARS-CoV, such as B- and T-cell epitopes with 23% and 16% similarities. A possible vaccine needs to be developed, keeping similar epitopes in mind. The vaccine must be capable of eliciting a strong immune response against the virus by activating B- and T-cells of the adaptive immune system, leading to the development of lifelong immunity against the virus [113].

Few studies suggest the role of antibodies (local and circulating) in protection against the coronavirus. Many studies demonstrated the shelf life of the antibodies, with some studies indicating it to be 5–6 months and others suggesting it to last at least a year. However, detailed studies need to be worked on to deduce the exact shelf life of the antibodies and understand the kinetics of antibody production [59,114]. The transfer of virus to neighbouring cells occurs directly, from cell–cell interaction without entry into the extracellular environment, considering T-cells act as the major immune cells which can control infection rather than the circulating antibodies [115].
7.5. Damage to the T-Lymphocytes Inflicted by COVID-19

The coronavirus is powerful in disabling the immune system by damaging T-lymphocytes. A study by Qin et al. [116] showed that 452 COVID-19 patients that were subjected to examination were divided into two groups based on the severity of their symptoms. There was an elevation in the number of infection-related biomarkers (i.e., procalcitonin, erythrocyte sedimentation rate, serum ferritin and C-reactive protein) and inflammatory cytokines (TNF-α, interleukin (IL)-2R and IL-6). There is a difference in the blood cell counts between the severe and the non-severe group, severe cases exhibit higher leukocyte and neutrophil counts with higher neutrophil to lymphocyte ratio and lower lymphocyte counts. The count of CD4+ cells was significantly reduced in severe cases, however, CD8+ and B-cell counts remained constant throughout. The reduction in T-lymphocytes is responsible for the compromised adaptive immune response against COVID-19 [116]. The high neutrophil to lymphocyte ratio is regarded as a characteristic of systemic inflammation and also as an indicator of bacterial infection [117]. High levels of pro-inflammatory cytokines and chemokines have been seen in the case of SARS-CoV and MERS-CoV infection which corroborates the high levels in case of infection from SARS-CoV-2 [86,87].

7.6. Gender-Based Differences in the Immune Response against COVID-19

There are gender-based differences as the human body perceives and responds differently to the COVID-19 virus. Worldwide data suggests that men suffer more from respiratory system diseases, including the ones caused by acute viral infections, while women are less prone to get infected by a virus, because of differences in their innate and adaptive immune systems. Sex hormones are known to regulate the innate defence mechanisms of the body. In the case of COVID-19 infection, gender differences influence the antiviral immune response, morbidity, transmission and pathogenesis. The estrogen hormone modulates the cytokine receptors as well as regulates the production of pro-inflammatory cytokines [118,119]. The cytokine release is responsible for the pathogenesis of COVID-19. Estrogen influences the immune response by binding to the estrogen receptor (ER α or β) [120], the ER-α receptor is found in all immune cells. The binding of estrogen to the ER-α receptor on immune cells results in their maturation and regulation and this binding also triggers the release of interferons which induces the activation of NK cells, exerting an immune protective effect [120,121]. Therefore, the immune system can be modulated by the estrogen hormone that binds to the ER α or β receptors. ER β has opposite effects to ER α and is involved in pro-inflammatory phenomena [122]. The loss of ER α in older women leads to immunosuppression, demonstrating that estrogen can protect against COVID-19 [123,124].

COVID-19 brings about this cytokine storm by interacting with the TLRs expressed on the surface of cells of the innate immune system including macrophages, mast cells and dendritic cells. This signalling cascade triggers the release of cytokines and chemokines by promoting vascularization. All these events collectively lead to a worsening of the patient, resulting in the manifestation of severe symptoms. It is well known that one of the X chromosomes in women is inactivated; however, the single activated X-chromosome is solely responsible for boosting the immune system in women. The X-chromosome regulates the response to viral infections by modulating the function of various receptors such as FOXP3, TLR7, TLR8, CD40L and CXCR3 resulting in their overexpression in women [125]. In addition, the comparison of the immune response in women with men may exhibit altered immune behaviour in terms of showing lower plasma viral loads, higher CD4+ T-cells and higher antibody levels with a longer shelf life [126]. Furthermore, sex hormones also govern the activation of immune cells by activating TLR7, causing the release of interferons which induces the antiviral state and alerts the immune cells about a possible viral infection that needs to be eliminated. The female sex hormones are more competent in activating the immune cells in comparison to males. The activation of immune cells results in the release of cytokines which are critical for activating the cytotoxic T-cells.
However, the cytokines are majorly responsible for inflammation, which is a characteristic feature of the aggravation of the COVID-19 disease. Even though the action of sex hormones in women results in a highly potent immune response, there are high chances of developing autoimmune and inflammatory disorders. The autoimmune response in the case of women is due to the overexpression of TLR7 but allows the system to be less sensitive to viral infections [127].

7.7. Contribution of Bioinformatics Tools for Designing Theranostics Approaches

As discussed above, the immune system is a complex system with innumerable cells acting in their unique way to attack potential invaders. Understanding the complex human system is a laborious and time-taking process. Computational biology comes to the rescue here, years of hard work have led to certain mathematical models which can predict immunogenic motifs in the target pathogen. These models can predict the interaction of B-cells or T-cells with specific regions of the pathogen. This progress in computational biology has enabled the scientific community to design a vaccine against viruses at record rates. If one knows the target genome sequence, researchers can get a potential vaccine candidate in a short period. Accumulation of mutations and natural selection over millions of years have strengthened the immune system in terms of diversity and adaptability allowing it to face any issues that may exist. Even though, there exists an in-depth understanding of the mouse immune system, but the human immune system is far from our understanding and needs to be studied step by step. Computational biology provides us with the relevant tools necessary for deciphering the immune system. This urgent need of decoding the immune system led to the development of a distinct field of “Systems Immunology”. It is a field of systems biology that uses mathematical approaches and computational methods to evaluate the interactions within cellular and molecular networks of the immune system [128]. Owing to the surge in COVID-19 cases, the focus of researchers has shifted towards understanding the evolution and pathogenesis of the virus. They are rigorously working to decode the sequence evolution at the genomic and proteomic level via phylodynamic and epidemiological models for designing potential drug candidates [129].

Bioimmuno-informatics is a branch of bioinformatics utilizing mathematical and computational approaches to develop immunological data and make predictions on immunity and disease pathogenesis. B-cells identify pathogen-specific epitopes and computational tools can be utilized for predicting the epitopes which can be recognized by immune cells. The process for modelling the immunogenic peptide sequences involves hunting the available COVID-19 sequence for all the open reading frames (ORFs), mapping and screening for expression.

In the case of conventional vaccine designing, the attenuated or disarmed pathogens must have been cultivated at a large scale to cater to the huge global population. The antigenicity of the agents must also be assessed. However, this is a time-consuming process and may take several years to complete. Therefore, there is an urgent need for methods that are fast, effective and safe for vaccine development. Recombinant DNA technology is being used in conjunction with BI tools for the validation and analysis of possible antigenic motifs. In addition to vaccine designing through antigen prediction using BI, drug repurposing of existing drugs is also another solution to solving problems in the COVID-19 situation since there are no approved antiviral drugs against COVID-19 [130].

7.8. Epitope Designing: The Process

The virus becomes infectious once it enters the host cell and viral entry is assisted by the SP [131]. Based on earlier studies on SARS and MERS viruses, this protein has emerged as highly immunogenic capable of inducing a strong immune response. Even though most studies have designed drugs based on the S-protein, mutations have emerged as the major block in their development. Therefore, it is important to have other options handy. Vaccines have been designed by incorporating other COVID-19 proteins such as N and M proteins,
accessory proteins, etc. Further, in a study by Sikora et al. [132], in silico docking studies were conducted for identifying possible antibody binding sites on a 4.1 million atom system containing a patch of the viral membrane with four full-length, fully glycosylated and palmitoylated S-proteins. They identified nine epitopes by generating a consensus epitope score which included the combined accessibility, rigidity, conservation and immunogenicity score by taking their product and ensuring the inclusion of high-scoring epitopes in all four studies. This study focussed on the importance of identifying small fragments of the antigen for vaccine design, without the need for working on full-length proteins. The significance of glycosylation of the surface of S-proteins was highlighted, glycans cover the surface of the S-proteins and provide steric hindrance and prevent the binding of neutralizing antibodies to the S-proteins. Both heavy and light glycosylation was analyzed in the study, and it has been found that light glycosylation sterically hindered the binding of neutralizing antibodies to the SP [132].

In another study by Sadat et al. [133], four protein models were generated through epitope prediction. Epitope prediction for vaccine design involves a couple of steps. The first step is the retrieval of the SARS-CoV-2 sequence, which is then subjected to the prediction of epitopes that can be the potential targets of B- and T-cells; the structure and properties of the epitopes are thoroughly analyzed for subsequent vaccine design. This process of vaccine designing is known as “Reverse Vaccinology” [133]. This in silico prediction and screening of vaccine candidates speeds up the process [134]. The four models which were analyzed for their antigenicity include RBD of the COVID-19 SP, a fusion protein composed of RBD, full-length M and N proteins of COVID-19, truncated spike protein and a fusion protein composed of truncated spike protein and full-length M and N proteins. A schematic of the four models is presented below in the Figure 4.

![Figure 4. Schematic representation of the four models.](image-url)

The results reveal that the fusion protein represented by model IV has the highest immunogenicity amongst the four models and consists of 24 highly immunogenic regions which are potential targets of B- and T-cells. The fused proteins are further being tested for their in vitro and in vivo efficacy. In another study by Ayman et al. [135], conclusive in silico studies indicate the evolutionary relationship between the SP of SARS-CoV-2 to that of SARS-CoV found in bats, showing a 96% sequence similarity. Their studies also suggest
that the SARS-CoV-2 is a virus that has been naturally created through natural selection from the SARS-CoV virus. They have also found a close evolutionary relationship between the ACE-2 receptor for the spike protein between humans and bats. Molecular docking studies by Chaudhury et al. revealed that the binding pockets for the spike proteins were found to be similar between bats and humans [61].

Furthermore, the pathogenesis of the virus is characterized by a release of pro-inflammatory cytokines such as interleukins and TNFs, triggered by the toll-like receptor signalling pathway. The SP of SARS-CoV-2 is known to bind to TLRs and induce the signalling cascade. If the interaction between the two is prevented by an inhibitor that competitively binds to TLRs, the spread of the viral infection could be prevented. Molecular docking studies revealed the high binding affinity of the spike protein for TLR-1,4 and 6, the interaction is mediated by the extracellular domains of the TLRs. They claim that the selective targeting of this ligand-receptor complex may pave a way for vaccine designing against COVID-19 [61]. In another study by Bhattacharya et al. [136], a peptide vaccine candidate has been analyzed in silico for its binding to TLR-5. The short length and helical structure of the peptide facilitated the binding with the TLR-5.

In a study by Lee et al. [137], computational validation studies were conducted to form a list of 48 potential immunogenic peptides that could be used for vaccine design against SARS-CoV-2. For this purpose, the open reading frames of SARS-CoV-2 were acquired from NCBI. The sequence similarity between the identified immunogenic peptides was available in the IEDB peptide database and was evaluated using the pairwise alignment function from the “biostrings v2.40.2 package”. Only those peptide sequences that exhibited a sequence similarity od more than three amino acid sequence were chosen for further scrutinization. The immunogenicity of the selected peptides was determined using an onlineipred prediction tool which relates scores of a peptide with its prospective recognition by a T-cell. Further, the binding affinity of a peptide towards MHC molecules was predicted using the NetMHC pan V4. The study also demonstrates the de novo search, of SARS-CoV-2 9-mer peptides which have a binding affinity towards HLA alleles found in Chinese and European populations, and the ability to recognize TCR using a new immunogenicity algorithm iPred, and 63 such peptides were identified [137].

7.9. Approaches for Vaccine Development

• Targeting the cellular immune system: For effective vaccine development against intracellular pathogens, the identification of protective antigens from thousands of candidates is a prerequisite. However, the relevant properties of the antigens which render protection to the endogenous pathogens against the cellular immune system are poorly characterized [138,139], delaying the process of vaccine development. Antigen abundance is an important property that can be exploited for this purpose [138,140]. The antigen expression profile varies from a few to millions of molecules per cell and the antigens which are highly expressed can be selectively tested for their immunogenicity [141,142]. In one such study by Rollenhagen et al., the selective recognition of a few highly expressing antigens by CD4+ cells led to the induction of a potent immune response which was shown by the in vivo selection of abundantly expressed antigens. Therefore, the selection of such abundant antigens may facilitate the development of effective vaccines against infectious diseases such as influenza, typhoid fever, COVID-19, etc. This process can be improved by studying the transcriptomic data from viral–host interactions [138].

• Pooling of highly immunogenic epitopes: The human serum consists of a range of polyclonal antibodies that may or may not correspond to immunogenic antigens. This is because a pathogen consists of several antigenic particles/epitopes which have originated from regions of the pathogens that were genetically evolved to be less immunogenic. This deceives the immune system into recognizing the non-immunogenic epitopes which undergo constant mutations. Even after having a memory of the antigens, the immune system fails on a second encounter because of the accumulation of
mutations in the epitopes. Selecting such antigens will ensure that the immune system can induce the production of antibodies specific to the pathogen. Therefore, pooling out the highly conserved epitopes of the virus which are seemingly non-immunogenic, and raising an antibody response against them in absence of the whole virus would enhance their immunogenicity and promise a robust immune response against the virus [90,143].

- **Computational Docking:** Designing vaccines can also be done by docking studies. The first step in the process of vaccine designing is the generation of crystal structures of the antigens and their respective antibodies. Once this task is accomplished, the next step is the in silico docking of both the structures in a bid to discover useful epitopes which are immunogenic. This approach was attempted by Kuntz et al. in 1982 [144]. In their study, they analyzed the binding geometries of different ligands and their respective receptors for steric overlapping by devising a docking program DOCK. This aided in identifying binding sites on the macromolecular surface. They studied the binding interactions between heme-myoglobin and thyroid hormone and pre-albumin. Since then, this field has had major reformations and is still flourishing at a rapid rate [144,145]. Some of the studies also involved the utilization of Ayurvedic plants *Piper longum* and *Ocimum sanctum* for the treatment of coronavirus by targeting ACE2 and TMPRSS2 receptor proteins [146].

- **Multi-subunit-based vaccines:** In a study by Dar et al., in silico studies were conducted on the spike protein of COVID-19 and multiple epitopes were identified for their ability to elicit a strong immune response. All of these epitopes were then combined via flexible GPGPG linkers along with a cholera toxin β subunit (CTB) sequence at the N-terminal joined by an EAAAK linker, as an adjuvant to form a multi-subunit vaccine candidate. This vaccine was then subjected to molecular docking studies with toll-like receptors to understand their interaction dynamics and to check for the immunogenicity of the multi-subunit vaccine against COVID-19. Quality assessment by the proSA web server showed a good match with experimentally resolved similar PDB structures. Ramachandran plot results revealed that 95% of residues were in favourable regions. Discotope and Ellipro servers revealed the competency of the vaccine in terms of possessing 27% and 52% epitopes recognized by the B-cells. The HADDOCK server showed that the binding between the protein clusters exhibited good stability and flexibility. However, there was an improvement in the HADDOCK scores when molecular refinements were applied to the docked protein complex. Moreover, the interactions between the protein clusters were also energetically favourable. Hence, in vitro studies must be performed to validate the docking results to further prove the effectiveness of the vaccine against COVID-19 [147].

Multi-epitope-based vaccines have gathered enormous attention recently. Singh et al. have designed a multi-epitope-based vaccine using four structural proteins of SARS-CoV-2 (S, M, N and E proteins), followed by in silico simulations for validating the immunogenic capability of the construct. Vaccine designing was accomplished by identifying the B- and T-cell and IFN-gamma epitopes on the four structural proteins. E glycoproteins scored the highest points in terms of antigenicity and emerged as a winner in this department. The generated vaccine possesses a molecular weight of 45.131 kDa. Other properties of the vaccine include a basic nature and high structural stability. The vaccine exhibited a prolonged shelf life which is adequate to trigger a robust immune response [148].

Vaccine improvement and further validation studies revealed the good quality of the model with 90% of the residues falling in the favourable region. Further docking studies have revealed that the vaccine exhibits good binding affinity towards the TLR-3 and TLR-4 which could prove to be instrumental in recognizing SARS-CoV-2 and mounting a robust innate and adaptive immune response against the antigen. Codon adaptation was adopted to enhance the expression levels of the candidate vaccine in the *E. Coli* expression vector. The vaccine was then cloned in a pET (+28) vector followed by simulation studies indicating that the vaccine will produce a strong primary and secondary immune response.
as predicted. Three subsequent injections were the threshold dosage required for inducing an adequate immune response. However, around 12 repeated injections of the candidate vaccine triggered an even stronger immune response. The levels of IFN-γ were also elevated, confirming the activation of an innate immune response [148].

In a study by Mitra et al., a multi-subunit-based vaccine has been designed that has been constructed using two subunits S1 and S2 of the spike glycoprotein of coronavirus in combination with a chimeric adjuvant. Spike glycoprotein has been known for its role in viral entry mediated by a dynamic interaction between the ACE-2 receptor found in the host cell. The studies demonstrate that when identifying antigenic epitopes against B- and T-cells if multiple servers are utilized in each case and run-on different algorithms, one must select those epitopes which have been validated by the results of the multiple servers [149–152]. This ensures that the selected epitopes will be effective in their task. By using this methodology, the predicted epitopes were found to have been mapped in a recent microarray study.

The predicted epitopes possessed parameters such as antigenicity, non-allergenicity, non-toxicity and a wide range across the spike glycoprotein. The epitopes also showed good sequence similarity with the sequence repository from Gujarat (QJC19491.1). They moved a step further by adding a chimeric adjuvant for maximizing the immune response against the constructs. The adjuvant comprises TLR-4 agonists and has been found to have sequence similarity with B-cell epitopes found in the IEDB server. The bits of adjuvant sequences were joined together by rigid sequences and the adjuvant sequences were combined with the epitopes using EAAAK as a linker. The arrangement of the adjuvant and the predicted epitopes was changed multiple times to come up with five vaccine constructs using ROBETTA modelling software which were characterized using the ProtParam parameters. They were predicted to be immunogenic because of the presence of ample B-cell epitopes and the capability to induce the interferon-gamma mediated immune response.

The constructs consist of cysteine bonds pointing towards their chemical stability. The molecular dynamic simulation was performed to study and validate the structure of the construct. The vaccine constructs were found to have a secondary structure consisting of loops and strand regions indicating its high dynamic stability. The modelled constructs were also evaluated for their half-life, using the Expasy ProtParam based on the assumptions of the PEST hypothesis (including the presence of the asparagine, lysine and glycine amino acid residues and a basic or neutral isoelectric point in stable proteins having a longer half-life) and two constructs, 4 and 5, were found to have the highest stability based on the assumptions and results of the Ramachandran plot, ERRAT-3D and Z-scores using a 100 ns molecular modelling simulation run.

Further, molecular dynamic simulations were carried out to study the interaction dynamic between the constructs and TLR-4, and contract 4 was found to have a high binding affinity for the receptor with a global energy score of −24.18 KJ/mol. The interaction between the construct and TLR-4 is driven by non-covalent interaction and this was visualized using the UCSF chimera tool (hydrophobic and hydrogen bonds) over the surface of the TLR-4. The residues which were involved in interaction were mapped through DimPlot. Post docking evaluation using MM/GBSA confirms the docked pose and the residues involved in this interaction [149].

8. Drug Repurposing

Drug repurposing is another sought-after strategy to combat COVID-19 infections. In this process, new therapeutic uses of an approved or investigational drug are intended for the treatment of other ailments. This strategy is safe, inexpensive and less time-consuming [153]. According to Wang et al. [153–156], drug–drug interaction studies and drug–drug–target interaction analysis are commonly used techniques for drug repurposing. Transcriptomics data can give insights into the gene expression profiles of SARS-CoV-2 during infection [154]. Therefore, tools such as Genotype-Tissue Expression (GTEx) [157,158]
immuno 2022, 2

and the program LINCS L1000 database [159,160] have been exploited for extracting the tissue-specific gene expression data.

The hunting of potential drug candidates has been eased by computational tools such as Drug Bank [161], ChEMBL19 [161] and ZINC1520 [162]. Bioinformatics tools are being extensively explored for this purpose, which is exhibiting promising results [157]. Various scoring docking functions such as the Glide Docking program, and molecular mechanical force field (MMFF) are being employed for shortlisting potential drugs and their optimization studies. All chemical thermodynamic integration (TI) and free energy perturbation (FEP) are popular techniques for structure-based drug discovery [154,163–166]. Nowadays, testing of two or more therapeutic agents is being utilized in combination to check their synergistic action against SARS-CoV-2 [167–169]. Multi-target directed ligands (MTDLs) are drugs that are an example of this approach, where two or more pharmaceutical agents with similar structures are joined together in a single complex [168,170,171].

Using molecular docking, the “best fit” conformation of a ligand to its receptor is simulated. In a study by Behera et al. [168] with 45 FDA-approved antiviral drugs were analyzed for their therapeutic potential against COVID-19. To this end, molecular docking of the drugs to three RNA dependent RNA Polymerase (RdRPs) was performed using Autodock software. Based on the highest ranking in terms of the binding energy, 10 antiviral agents outperformed and Paritaprevir and Grazoprevir were among those ligands. Even though Peritaprevir had a higher binding affinity towards the receptor, Grazoprevir was chosen for further studies because of its ability to bind to key residues in the ACE-2 receptor and the RdRP. Molecular dynamics and Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA) analysis studies showed that the complex formation between the ligand and the target proteins was highly stable [168].

**Targeting Viral Replication Proteins for Drug Repurposing**

In a study by Wang et al. [154], two-step hierarchical virtual screenings were carried out on 2201 approved drugs (Drug Bank) for repurposing against SARS-CoV-2. They have tested the efficacy of these drugs against the SARS-CoV-2 protease which is critical for viral replication. This process involved three major steps:

(a) Enriching the drug candidates using glide software;
(b) Evaluation of the docking hits using the MM-PBSA-WSAS method;
(c) Selection of the drug candidates based on their binding energies.

Based on this analysis, five potential drug candidates were identified which showed inhibitory properties against the protease enzyme namely carfilzomib, tetracycline, valrubicin, lopinavir and elbasvir. RNA viruses undergo rapid mutations; however, active sites of essential proteins remain evolutionarily conserved, and thus targeting these regions is an effective strategy for defeating the virus [154].

In another study by Balasubramanium et al., three evolutionarily conserved proteins, namely RdRP, papain-like proteinase and helicase, were targeted for testing the antiviral ability of ~3300 investigational drugs and 54 FDA-approved drugs. These proteins are critical for viral replication and blocking either one or all of them could prove to be fatal to the COVID-19 virus [172–175].

The structural models of the above proteins developed by Zhang et al. [176] were employed and the regions in these proteins which could act as binding sites for the drugs were predicted using Discovery Studio Suite. Molecular dynamic simulations were performed for analyzing the stability of the binding pockets over time and the results confirmed their high stability. These predicted binding pockets were screened against the 3300 investigational and 54 FDA-approved drugs. Screening results showed that the drug elbasvir is a potent agent which had a broad binding affinity for the three target proteins [174]. However, the authors asserted that the drug is highly reactive and it could influence off-target effects as well [177], but the dose at which it is administered is not reported to induce any serious side effects [178].
The reason for the strong affinity of the drug toward the three target proteins is that the drug majorly interacts with positively charged proteins and the release of the viral particles from the host cell is governed by the assembly of viral protein around its genetic material (RNA). This interaction occurs via electrostatic binding of the positively charged proteins with the negatively charged RNA backbone. This promotes the binding of the drug to the binding pockets [174].

9. Phage Display Techniques for Understanding the Molecular Dynamics of Viral Pathogenesis

Bacteriophages are viruses that infect bacterial cells. Over the past three decades, phages are being extensively utilized for the display of certain peptide sequences as fusion proteins on the surface of the bacteriophage protein coat [179]. This technique was used for the first time to establish the linkage between phenotype and genotype and came to be known as phage display [180]. As discussed in the previous sections, the SARS-CoV-2 virus has undergone several mutations, giving rise to viral strains with higher infectivity and immune evasion capabilities. In addition, viral mutations which confer immune resistance and lead to alternative structures of the spike protein which allow the virus to attach to a varied number of cellular receptors are selected throughout evolution [181–185]. Other receptors which may serve as potential entry sites for the virus include the integrin-binding motif which mediates entry through the binding of integrins resulting in the activation of transforming growth factor (TGF)-β [186]. In addition, basigin CD147 or the tyrosine-protein kinase receptor are also other receptors that facilitate the viral binding and entry into the host [187,188]. To understand the mechanisms which strengthen viral pathogenesis and to study the dynamics of immune evasion recombinant filamentous phages may be used. Phage libraries consist of distinct phage particles which display fused protein on the N-terminal of their p-8 major coat protein. The phage bears 4000 copies of the p-8 coat protein which can be used for the display of an array of peptides whose functionalities are influenced by their interactions with the neighbouring peptides and the body of the phage particle [189,190]. Pentrenko et al. [191] have discussed peptide sequences that resemble the receptor-binding domains of the viral spike protein known as “mimotopes” which are made to express on the surface of the phage particles. These mimotopes are effective probing tools for understanding the molecular dynamics of viral infection. They may be exploited to exhibit specificity towards the attachment proteins of SARS-CoV-2 for their application in vaccine designing.

In their study, they have developed phage mimotopes of the SARS-CoV-2 Spike S1 protein through phage display and molecular mimicry concepts, termed here “phage mimicry”, by using bioinformatics methods. Molecular mimicry is used to understand the cross-reactivity of certain antigenic peptides towards the conformational peptides or amino acid sequences of a receptor which may cause an unwanted autoimmune response [192,193]. A panel of closely related phage mimotopes mimicking the receptor-binding sites on the surface of fibroblast growth factor (FGF1), NRP1 and the SARS-CoV-2 spike protein have been identified, and their binding to FGFR3 was shown by ELISA and confirmed by molecular modelling. It was found that the binding AA clusters of NRP-1 bear striking resemblance to the FGF-1 and the receptor-binding domain of the SARS-CoV-2 spike protein. It was revealed that the phage mimotopes that exhibited similar amino acid patterns to the RBD of spike protein, which mediate interactions with the FGFR3, were not found in the SARS-CoV variant [194]. It was deduced that these mutated residues have granted evolutionary fitness to the new variants of the SARS-CoV-2 virus allowing it to attain a more aggressive phenotype [195]. The phage EDYSELVSQ has been isolated by affinity selection from Calu-3 cells which is a cell line of the non-small cell lung cancer that overexpress human growth factor receptors and other important cellular receptors. This cell line is used for studying the pathogenesis of the SARS-CoV and SARS-CoV-2 viruses in cell culture [196]. The findings suggest that the evolution of the viral strains in the human population may occur either through immune evasion or through the exchange of
viral receptors and co-receptors [197,198]. If the molecular pathways of viral pathogenesis are thoroughly understood using the bioinformatics tools, the conceptualization of these mechanisms will open new avenues for the designing of vaccine candidates which are effective against mutant viral strains. Computational methods can be used to determine the purpose of the amino acid changes occurring in circulating viral variants and help in the prediction of amino acid residues that may undergo mutations shortly to pave way for new viral mutants. Popular computational tools that are employed to study the effect of amino acid mutations on the protein–protein interactions include Single Amino Acid Mutation based change in Binding free Energy (SAAMBE) [199] and BindProfX [197,200].

10. Artificial Intelligence (AI) Based Vaccine Designing and Drug Repurposing against SARS-CoV-2 Virus

The past two decades have become a testimony to the popularity of AI tools in the discovery of potential vaccine candidates against viral infections. AI tools aid in the analysis of huge amounts of data and allow accurate predictions about the data [201]. The general concept behind AI is the development of techniques that are capable of mimicking the cognitive abilities/functions of the brain for problem-solving and learning [202]. AI is divided into two main branches namely,

- Machine learning (ML): ML allows for the generation of models which analyze and learn from the available data to identify patterns and derive inferences from previously unseen data [203]. Some of the popular ML algorithms include support vector machines, random forest classifiers, k-means, hierarchical clustering, etc., and recently artificial neural networks (ANN) [204].
- Deep learning (DL): On the other hand, deep learning allows for automatic feature extraction from raw data [205]. DL allows for a deep analysis of the data using advanced ANN-based ML algorithms by using multilayered processing units [206].

AI algorithms can expedite the process of drug discovery against COVID-19. Studies have been conducted where DL-based algorithms such as DeepCE have been used for the deep analysis of potential drug candidates that could be repurposed against COVID-19 infection [207]. Furthermore, another DL-based drug target model has been developed in another study to repurpose drug compounds that have the potential to target SARS-CoV-2 proteins [5]. In another study by Wallach et al., AtomNet R deep convolutional neural network technology has been employed to develop broad-spectrum antivirals by targeting protein binding sites which are evolutionarily conserved across coronavirus species [208]. In another study by Ton et al., 1000 SARS-CoV-2 protease inhibitors were identified by creating and utilizing the Deep Docking (DD) network technology approach [209]. AI is also extensively being explored for vaccine development against COVID-19, by developing programs such as MAREA (major histocompatibility complex analysis with recurrent integrated architecture) and MoDec that predicts antigen presentation [210–212]. Furthermore, AI tools have also been utilized to analyze the presentation of viral proteins to MHC molecules from patients to understand the mechanisms which confer immunity in the host.

In another study by Ong et al., six proteins that could serve as targets for vaccines were discovered in the SARS-CoV-2 proteome using a combination of reverse vaccinology and machine learning [204,213]. AI-based vaccine designing and drug repurposing have led to a paradigm shift in developing therapeutic strategies against COVID-19.

11. Conclusions

The emergence of the COVID-19 pandemic is currently in an extreme global massacre state and the condition is worsening day by day catastrophically. In this scenario of a high mortality rate, there is a requirement of understanding immunogenic triggering responses against coronavirus. There are various diagnostic methods devised with different infecting tissues. However, the virus is rapidly mutating, leading to the emergence of variants such as B.1.1.7 B.1.351/S01.YV2, B.1.1.248/B1.1.28/P1 and the Cluster 5. Therefore, regular sequencing of the viral genomes which cause failures of the diagnostics tests is an effective
strategy to identify the sites of mutations that might affect the target binding in diagnostic tests. ACE-2 is reported as the first target protein that facilitates viral entry into the host. Once it enters, the virus overlaps the host machinery and undergoes replication, translation and assembly. All four virus glycoproteins are essential in the transmission of the disease. The B- and T-lymphocytes get activated on their action and initiate a signalling cascade to fight against the virus. However, an impaired immune response, due to a reduction in the T-lymphocytes, contributes to the severity of COVID-19. Furthermore, the release of inflammasomes, DAMPs, TLRs and various pro-inflammatory cytokines play a critical role in escalating the symptoms of COVID-19.

With time, bioinformatics and machine learning have taken the field to the next level by predicting targets and interactions. Various studies demonstrated the use of BI and machine learning tools to study the evolutionary relationship between two viruses, vaccine development, pooling of highly immunogenic epitopes, drug repurposing, etc. establishing their potential in the fight against COVID-19.

Author Contributions: D.Y. and M.S. conceptualized, wrote and edited the manuscript and performed the literature survey. S.A. ideated the scheme, performed artwork and drafted the manuscript. P.P., D.J., V.A., P.K.D., S.M., R., A.D., N.K.J. and K.K.K. edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Zheng, J. SARS-CoV-2: An Emerging Coronavirus that Causes a Global Threat. Int. J. Biol. Sci. 2020, 16, 1678–1685. [CrossRef] [PubMed]
2. Liu, D.X.; Liang, J.Q.; Fung, T.S. Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). Enycl. Virol. 2021, 428–440.
3. Malik, Y.A. Properties of Coronavirus and SARS-CoV-2. Malays. J. Pathol. 2020, 42, 3–11.
4. Schoeman, D.; Gordon, B.; Fielding, B.C. Pathogenic Human Coronaviruses. In Reference Module in Biomedical Sciences; Elsevier: Amsterdam, The Netherlands, 2021.
5. Beck, B.R.; Shin, B.; Choi, Y.; Park, S.; Kang, K. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. Comput. Struct. Biotechnol. J. 2020, 18, 784–790. [CrossRef] [PubMed]
6. Kowalik, M.M.; Trzonkowski, P.; Łasińska-Kowara, M.; Mital, A.; Smiatacz, T.; Jaguszewski, M. COVID-19—Toward a comprehensive understanding of the disease. Cardiol. J. 2020, 27, 99–114. [CrossRef] [PubMed]
7. Bhattacharya, S.; Sharma, P.; Mathur, H.; Rasheed, H.; Singh, S.; Rajput, G.; Agnihotri, S.; Nirmal, P.; Kaur, S. Recent apprise on coronavirus and its terrible insinuations. Virodisease 2020, 31, 121–127. [CrossRef]
8. Bhattacharya, M.; Sharma, A.R.; Patra, P.; Ghosh, P.; Sharma, G.; Patra, B.C.; Saha, R.P.; Lee, S.S.; Chakraborty, C. A SARS-CoV-2 vaccine candidate: In-silico cloning and validation. Inform. Med. Unlocked 2020, 20, 100394. [CrossRef]
9. Woo, P.C.; Lau, S.K.; Lam, C.S.; Lai, K.K.; Huang, Y.; Lee, P.; Luk, G.S.; Dyrtling, K.C.; Chan, K.H.; Yuen, K.Y. Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. J. Virol. 2009, 83, 908–917. [CrossRef] [PubMed]
10. V’kovski, P.; Kratzel, A.; Steiner, S.; Stalder, H.; Thiel, V. Coronavirus biology and replication: Implications for SARS-CoV-2. Nat. Rev. Microbiol. 2021, 19, 155–170. [CrossRef] [PubMed]
11. Alлуwaimi, A.M.; Alshubaith, I.H.; Al-Ali, A.M.; Abohelaika, S. The Coronaviruses of Animals and Birds: Their Zoonosis, Vaccines, and Models for SARS-CoV and SARS-CoV2. Front. Vet. Sci. 2020, 7, 582287. [CrossRef] [PubMed]
14. Woo, P.C.; Huang, Y.; Lau, S.K.; Yuen, K.Y. Coronavirus genomics and bioinformatics analysis. *Viruses* **2010**, *2*, 1804–1820. [CrossRef] [PubMed]
15. Harvey, W.T.; Carabelli, A.M.; Jackson, B.; Gupta, R.K.; Thomson, E.C.; Harrison, E.M.; Ludden, C.; Reeve, R.; Rambaut, A.; Peacock, S.J.; et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat. Rev. Microbiol.* **2021**, *19*, 409–424. [CrossRef]
16. Duchene, S.; Featherstone, L.; Haritopoulou-Sinanidou, M.; Rambaut, A.; Lemey, P.; Baele, G. Temporal signal and the phylogenomic threshold of SARS-CoV-2. *Virology* **2020**, *6*, vea061. [CrossRef]
17. Worobey, M.; Pekar, J.; Larsen, B.B.; Nelson, M.I.; Hill, V.; Joy, J.B.; Rambaut, A.; Suchard, M.A.; Wertheim, J.O.; Lemey, P. The emergence of SARS-CoV-2 in Europe and North America. *Science* **2020**, *370*, 564–570. [CrossRef]
18. Banoun, H. Evolution of SARS-CoV-2: Review of Mutations, Role of the Host Immune System. *Nephrin* **2021**, *145*, 392–403. [CrossRef]
19. Muth, D.; Cormann, V.M.; Roth, H.; Binger, T.; Dijkman, R.; Gottula, L.T.; Glozo-Rausch, F.; Balboni, A.; Battilani, M.; Rihtarić, D.; et al. Attenuation of replication by a 29 nucleotide deletion in SARS-coronavirus acquired during the early stages of human-to-human transmission. *Sci. Rep.* **2018**, *8*, 15177. [CrossRef]
20. Cosar, B.; Karagulleoglu, Z.Y.; Unal, S.; Ince, A.T.; Uncuoglu, D.B.; Tuncer, G.; Kilinc, B.R.; Ozkan, Y.E.; Ozkoc, H.C.; Demir, I.N.; et al. SARS-CoV-2 Mutations and their Viral Variants. *Cytokine Growth Factor Rev.* **2022**, *63*, 10–22. [CrossRef]
21. Viana, R.; Moyo, S.; Amoako, D.G.; Tegally, H.; Scheepers, C.; Althaus, C.L.; Anyaneji, U.J.; Bester, P.A.; Boni, M.F.; Chand, M.; et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. *Nature* **2022**, *603*, 679–686. [CrossRef]
22. WHO. COVID-19 New Variants: Knowledge Gaps and Research. WHO R&D Blueprint: Geneva, Switzerland, 2021.
23. Naveca, F.; Nascimento, V.; Souza, V.; Corado, A.; Nascimento, F.; Silva, G.; Costa, Á.; Duarte, D.; Pessoa, K.; Gonçalves, L.; et al. Phylogenetic Relationship of SARS-CoV-2 Sequences from Amazonas with Emerging Brazilian Variants Harboring Mutations E484K and N501Y in the Spike Protein. 2021. Available online: https://www.virological.org/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/588 (accessed on 26 April 2022).
24. Zhang, W.; Davis, B.D.; Chen, S.S.; Sincuri Martinez, J.M.; Plummer, J.T.; Vail, E. Emergence of a Novel SARS-CoV-2 Variant in Southern California. *JAMA* **2021**, *325*, 1324–1326. [CrossRef] [PubMed]
25. Tian, D.; Sun, Y.; Xu, H.; Ye, Q. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *J. Med. Virol.* **2022**, *94*, 2376–2383. [CrossRef] [PubMed]
26. Kar, S. COVID-19 Alert: WHO Says New Virus Strain ‘XE’ Could be Most Contagious So Far. 2022. Available online: https://www.hindustantimes.com/world-news/covid19-alert-who-says-new-virus-strain-xe-could-be-most-contagious-so-far-10164889465415.html (accessed on 26 April 2022).
27. Zheng, J.; Zhang, Y.; Kang, J.-Y.; Chen, S.; He, Y.; Han, B.; Liu, M.-F.; Lu, L.; Li, L.; Yi, Z.; et al. Potential transmission chains of variant B.1.1.7 and co-mutations of SARS-CoV-2. *Cell Discov.* **2021**, *7*, 44. [CrossRef] [PubMed]
28. Tegally, H.; Wilkinson, E.; Giovanetti, M.; Iranzadeh, A.; Fonseca, V.; Gandhari, J.; Doolabh, D.; Pillay, S.; San, E.J.; Msomi, N.; et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature* **2021**, *592*, 438–443. [CrossRef] [PubMed]
29. Negi, S.S.; Schein, C.H.; Braun, W. Regional and temporal coordinated mutation patterns in SARS-CoV-2 spike protein revealed by a clustering and network analysis. *Sci. Rep.* **2022**, *12*, 1128. [CrossRef] [PubMed]
30. Yang, X.-J. SARS-COV-2 Y variant acquires spike P681H or P681R for improved viral fitness. *bioRxiv* **2021**.
31. Kemp, S.A.; Collier, D.A.; Dari, R.P.; Ferreira, I.A.T.M.; Gayed, S.; H comunhoff, C.; Rees-Spear, C.; Lumb, I.U.; et al. SARS-CoV-2 Spike E protein evolution during treatment of chronic infection. *Nature* **2021**, *592*, 277–282. [CrossRef]
32. Bal, A.; Destras, G.; Gaymard, A.; Bouscambert-Duchamp, M.; Valette, M.; Escuret, V.; Frobert, E.; Billaud, G.; Trouillet-Assant, S.; Cheynet, V.; et al. Molecular characterization of SARS-CoV-2 in the first COVID-19 cluster in France reveals an amino acid deletion in nsp2 (Asp268del). *CMI* **2020**, *26*, 960–962. [CrossRef]
33. Robson, F.; Khan, K.S.; Le, T.K.; Paris, C.; Demirbag, S.; Barfuss, P.; Rocchi, P.; Ng, W.L. Coronavirus RNA Proofreading: Molecular Basis and Therapeutic Targeting. *Mol. Cell* **2020**, *79*, 710–727. [CrossRef]
34. Fehr, A.R.; Perlman, S. Coronaviruses: An overview of their replication and pathogenesis. *Methods Mol. Biol.* **2015**, *1282*, 1–23. [CrossRef] [PubMed]
35. Jacob, C.O. On the genetics and immunopathogenesis of COVID-19. *J. Clin. Immunol.* **2020**, *220*, 108591. [CrossRef] [PubMed]
36. Banerjee, A.; Kulcsar, K.; Misra, V.; Frieman, M.; Mossman, K. Bats and Coronaviruses. *J. Clin. Immunol.* **2020**, *108591*. [CrossRef]
37. Astuti, I.; Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes Metab. Syndr.* **2020**, *14*, 407–412. [CrossRef] [PubMed]
38. Harapan, H.; Itoh, N.; Yufika, A.; Winardi, W.; Keam, S.; Te, H.; Megawati, D.; Hayati, Z.; Wagner, A.L.; Mudatsir, M. Coronavirus disease 2019 (COVID-19): A literature review. *J. Infect. Public Health* **2020**, *13*, 667–673. [CrossRef]
39. Mathuria, J.P.; Yadav, R. Rajkumar, Laboratory diagnosis of SARS-CoV-2—a review of current methods. *J. Infect. Public Health* **2020**, *13*, 901–905. [CrossRef]
40. Deng, H.; Jayawardena, A.; Chan, J.; Tan, S.M.; Alan, T.; Kwan, P. An ultra-portable, self-contained point-of-care nucleic acid amplification test for diagnosis of active COVID-19 infection. *Sci. Rep.* **2021**, *11*, 15176. [CrossRef]
41. FDA. OPTI SARS-CoV-2 RT-PCR Test. 2020. Available online: https://www.fda.gov/media/137739/download (accessed on 26 April 2022).
42. WHO. Protocol: Real-Time RT-PCR Assays for the Detection of SARS-CoV-2; Institut Pasteur: Paris, France, 2019.
70. Zoabi, Y.; Deri-Rozov, S.; Shomron, N. Machine learning-based prediction of COVID-19 diagnosis based on symptoms. npj Digit. Med. 2021, 4, 3. [CrossRef] [PubMed]
71. Ahsan, M.M.; Nazim, R.; Siddique, Z.; Huebner, P. Detection of COVID-19 Patients from CT Scan and Chest X-ray Data Using Modified MobileNetV2 and LIME. Healthcare 2021, 9, 1099. [CrossRef] [PubMed]
72. Siddiq, Z.; Harif, M.; Dwivedi, k.K.; Chopra, K.K. Benefits and limitations of serological assays in COVID-19 infection. Indian J. Tuberc. 2020, 67 (Suppl. S4), S163–S166. [CrossRef]
73. Chantal Babb de Villiers, L.B.; Cook, S.; Janus, J. SARS-CoV-2 Variants. 2021. Available online: https://www.finddx.org/wp-content/uploads/2021/03/COVID-variants-report-FINAL-12MAR2021.pdf (accessed on 26 April 2022).
74. MacKay, M.J.; Hooker, A.C.; Afshinnekoo, E.; Salit, M.; Kelly, J.; Feldstein, J.V.; Haft, N.; Schenkel, D.; Nambi, S.; Cai, Y.; et al. The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. Nat. Biotechnol. 2020, 38, 1021–1024. [CrossRef]
75. Artesi, M.; Bontems, S.; Gobbel, P.; Franckh, M.; Maes, P.; Boreux, R.; Meex, C.; Melin, P.; Hayette, M.P.; Bours, V.; et al. A Recurrent Mutation at Position 26340 of SARS-CoV-2 Is Associated with Failure of the E Gene Quantitative Reverse Transcription-PCR Utilized in a Commercial Dual-Target Diagnostic Assay. J. Clin. Microbiol. 2020, 58, e01598–20. [CrossRef]
76. WHO. Transmission of SARS-CoV-2: Implications for Infection Prevention Precautions. 2020. Available online: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions (accessed on 26 April 2022).
77. Hamming, I.; Timens, W.; Bulthuis, M.L.; Lely, A.T.; Navis, G.; van Goor, H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. J. Pathol. 2004, 203, 631–637. [CrossRef]
78. Lodish, H.; Berk, A.; Zipursky, L.; Matsudaira, P.; Baltimore, D.; Darnell, J. Molecular Cell Biology; W. H. Freeman: New York, NY, USA, 2000; Volume 4.
79. Janeway, C.A. Immunobiology: The Immune System in Health and Disease; Garland Science: New York, NY, USA, 2001; Volume 5.
80. Dottiwa, F.; Mulik, S.; Polidoro, R.B.; Ansara, J.A.; Feldstein, J.V.; Haft, N.; Schenkel, D.; Nambi, S.; Cai, Y.; et al. The COVID-19 XPRIZE and the need for scalable, fast, and widespread testing. Nat. Biotechnol. 2020, 38, 1021–1024. [CrossRef]
81. Le Page, C.; Génin, P.; Baines, M.G.; Hiscott, J. Interferon activation and innate immunity. Rev. Immunogenet. 2000, 2, 374–386.
82. Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. Molecular Biology of the Cell; Garland Science: New York, NY, USA, 2002; Volume 4.
83. Hosseini, A.; Hashemi, V.; Shomali, N.; Asghari, F.; Gharibi, T.; Akbari, M.; Gholizadeh, S.; Jafari, A. Innate and adaptive immune responses against coronavirus. Biomed. Pharm. 2020, 132, 110859. [CrossRef]
84. Catanzaro, M.; Fagioli, F.; Racchi, P.; Corsini, E.; Govoni, S.; Lanni, C. Immune response in COVID-19: Addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct. Targ. Ther. 2020, 5, 84. [CrossRef] [PubMed]
85. Tay, M.Z.; Poh, C.M.; Rénia, L.; MacAry, P.A.; Ng, L.F.P. The trinity of COVID-19: Immunity, inflammation and intervention. Nat. Rev. Immunol. 2020, 20, 363–374. [CrossRef] [PubMed]
86. Coperchini, F.; Chiovato, L.; Croce, L.; Magni, F.; Rotondi, M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 2020, 53, 25–32. [CrossRef] [PubMed]
87. Costela-Ruiz, V.J.; Illescas-Montes, R.; Puerta-Puerta, J.M.; Ruiz, C.; Melguizo-Rodriguez, L. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Cytokine 2019, 119, 64–72. [CrossRef]
88. Subbarao, K.; Mahanty, S. Respiratory Virus Infections: Understanding COVID-19. Immunity 2020, 52, 905–909. [CrossRef]
89. Verdecchia, P.; Cavallini, C.; Spanevello, A.; Angeli, F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur. J. Intern. Med. 2020, 76, 14–20. [CrossRef]
90. Garcia, I.F. Immune Response, Inflammation, and the Clinical Spectrum of COVID-19. Front. Immunol. 2020, 11, 1441. [CrossRef]
91. Shah, V.K.; Firmal, P.; Alam, A.; Ganguly, D.; Chattopadhyay, S. Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. Front. Immunol. 2021, 11, 1949. [CrossRef]
92. Felsenstein, S.; Herbert, J.A.; McNamara, P.S.; Hedrich, C.M. COVID-19: Immunology and treatment options. Clin. Immunol. 2020, 215, 108448. [CrossRef]
93. Fung, T.S.; Liu, D.X. Human Coronavirus: Host-Pathogen Interaction. Annu. Rev. Microbiol. 2019, 73, 529–557. [CrossRef]
94. Ramasamy, S.; Subbian, S. Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis. Clin. Microbiol. Rev. 2021, 34, e00299–20. [CrossRef] [PubMed]
95. Del Valle, D.M.; Kim-Schulze, S.; Huang, H.-H.; Beckmann, N.D.; Nirenberg, S.; Wang, B.; Lavin, Y.; Swartz, T.H.; Madduri, D.; Stock, A.; et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat. Med. 2020, 26, 1636–1643. [CrossRef] [PubMed]
96. Carty, M.; Guy, C.; Bowie, A.G. Detection of Viral Infections by Innate Immunity. Biochim. Biophys. Acta 2020, 1829, 854–865. [CrossRef] [PubMed]
156. Breckenridge, A.; Jacob, R. Overcoming the legal and regulatory barriers to drug repurposing. Nat. Rev. Drug Discov. 2019, 18, 1–2. [CrossRef] [PubMed]

157. Galindez, G.; Matschinske, J.; Rose, T.D.; Sadegh, S.; Salgado-Albarrán, M.; Spáth, J.; Baumbach, J.; Pauling, J.K. Lessons from the COVID-19 pandemic for advancing computational drug repurposing strategies. Nat. Comput. Sci. 2021, 1, 33–41. [CrossRef]

158. The Genotype-Tissue Expression (GTEx) project. Nat. Gen. 2013, 45, 580–585. [CrossRef]

159. Musa, A.; Tripathi, S.; Dehmer, M.; Emmert-Streib, F. L1000 Viewer: A Search Engine and Web Interface for the LINCS Data Repository. Front. Gen. 2019, 10, 557. [CrossRef]

160. Duan, Q.; Flynn, C.; Niepel, M.; Hafner, M.; Mühlich, J.L.; Fernandez, N.F.; Rouillard, A.D.; Tan, C.M.; Chen, E.Y.; Golub, T.R.; et al. LINCS Canvas Browser: Interactive web app to query, browse and interrogate LINCS L1000 gene expression signatures. Nucleic Acids Res. 2014, 42, W449–W460. [CrossRef]

161. Wishart, D.S.; Feunang, Y.D.; Guo, A.C.; Lo, E.J.; Marcu, A.; Grant, J.R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; et al. DrugBank 5.0: A major update to the DrugBank database for 2018. Nucleic Acids Res. 2018, 46, D1074–D1082. [CrossRef]

162. Sterling, T.; Irwin, J.J. ZINC 15–Ligand Discovery for Everyone. J. Chem. Inf. Model. 2015, 55, 2324–2337. [CrossRef]

163. Reddy, M.R.; Reddy, C.R.; Rathore, R.S.; Erion, M.D.; Aparoy, P.; Reddy, R.N.; Reddanna, P. Free energy calculations to estimate ligand-binding affinities in structure-based drug design. Curr. Pharm. Des. 2014, 20, 3323–3337. [CrossRef]

164. Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Repasky, M.P.; Knoll, E.H.; Shelley, M.; Perry, J.K.; et al. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J. Med. Chem. 2004, 47, 1739–1749. [CrossRef] [PubMed]

165. Wang, J.; Morin, P.; Wang, W.; Kollman, P.A. Use of MM-PBSA in reproducing the binding free energies to HIV-1 RT of TIBO derivatives and predicting the binding mode to HIV-1 RT of efavirenz by docking and MM-PBSA. J. Am. Chem. Soc. 2001, 123, 5221–5230. [CrossRef] [PubMed]

166. Wang, J.; Kang, X.; Kuntz, I.D.; Kollman, P.A. Hierarchical database screenings for HIV-1 reverse transcriptase using a pharmacophore model, rigid docking, solvation docking, and MM-PB/SA. J. Med. Chem. 2005, 48, 2432–2444. [CrossRef] [PubMed]

167. Bang, S.; Son, S.; Kim, S.; Shin, H. Disease Pathway Cut for Multi-Target drugs. BMC Bioinform. 2019, 20, 74. [CrossRef] [PubMed]

168. Behera, S.K.; Vhora, N.; Contractor, D.; Shard, A.; Kumar, D.; Kalia, K.; Jain, A. Computational drug repurposing study elucidating molecular aspects concerning the use of the SARS-CoV-2 Receptor Binding Domain as a Target for Preventive Vaccines. Expert Opin. Drug Discov. 2021, 17, 923–929. [CrossRef] [PubMed]

169. Agostini, M.L.; Andres, E.L.; Sims, A.C.; Graham, R.L.; Sheahan, T.P.; Lu, X.; Smith, E.C.; Case, J.B.; Feng, J.Y.; Jordan, R.; et al. Multi-Target Directed Ligands (MTDLs) Binding the Molecular Interactions With the Renin-Angiotensin System. Int. J. Mol. Sci. 2021, 22, 7570. [CrossRef]

170. Abatematteo, F.S.; Niso, M.; Contino, M.; Leopoldo, M.; Abate, C. Multi-Target Directed Ligands (MTDLs) Binding the σ2 Receptor as Promising Therapeutics: State of the Art and Perspectives. Int. J. Mol. Sci. 2021, 22, 6359. [CrossRef]

171. Bobrowski, T.; Chen, L.; Eastman, R.T.; Itkin, Z.; Shinn, P.; Chen, C.; Guo, H.; Zheng, W.; Michael, S.; Simeonov, A.; et al. Discovery of Synergistic and Antagonistic Drug Combinations against SARS-CoV-2 In Vitro. bioRxiv 2020. [CrossRef]

172. Jacobson, I.M.; Lawitz, E.; Kwo, P.Y.; Hézode, C.; Peng, C.-Y.; Howe, A.Y.M.; Hwang, P.; Wahl, J.; Robertson, M.; Barr, E.; et al. Safety and Efficacy of Elbasvir/Grazoprevir in Patients With Hepatitis C Virus Infection and Compensated Cirrhosis: An Integrated Analysis. Gastroenterology 2017, 152, 1372–1382.e2. [CrossRef] [PubMed]

173. Smith, G.P.; Petrenko, V.A. Phage Display. Chem. Rev. 1997, 97, 391–410. [CrossRef] [PubMed]

174. Burton, D.R. Phage display. Immunotechnology 1995, 1, 87–94. [CrossRef]

175. Valdes-Balbin, Y.; Santana-Mederos, D.; Paquet, F.; Fernandez, S.; Climent, Y.; Chiodo, F.; Rodriguez, L.; Sanchez Ramirez, B.; Leon, K.; Hernandez, T.; et al. Molecular Aspects Concerning the Use of the SARS-CoV-2 Receptor Binding Domain as a Target for the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. mBio 2018, 9, e00211-18. [CrossRef]

176. Agis-Torres, A.; Söhlhuber, M.; Fernandez, M.; Sanchez-Montero, J.M. Multi-Target-Directed Ligands and other Therapeutic Strategies in the Search of a Real Solution for Alzheimer’s Disease. Curr. Neuropharmacol. 2014, 12, 2–36. [CrossRef]

177. Balasubramanian, S.; Rao, N.M.; Goenka, A.; Roderick, M.; Ramanan, A.V. Coronavirus Disease 2019 (COVID-19) in Children—What We Know So Far and What We Do Not. Indian Pediatr. 2020, 57, 435–442. [CrossRef]

178. Agostini, M.L.; Andres, E.L.; Sims, A.C.; Graham, R.L.; Sheahan, T.P.; Lu, X.; Smith, E.C.; Case, J.B.; Feng, J.Y.; Jordan, R.; et al. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. mBio 2018, 9, e00211-18. [CrossRef]

179. Agostini, M.L.; Andres, E.L.; Sims, A.C.; Graham, R.L.; Sheahan, T.P.; Lu, X.; Smith, E.C.; Case, J.B.; Feng, J.Y.; Jordan, R.; et al. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. mBio 2018, 9, e00211-18. [CrossRef]

180. Burton, D.R. Phage display. Immunotechnology 1995, 1, 87–94. [CrossRef]

181. Valdes-Balbin, Y.; Santana-Mederos, D.; Paquet, F.; Fernandez, S.; Climent, Y.; Chiodo, F.; Rodriguez, L.; Sanchez Ramirez, B.; Leon, K.; Hernandez, T.; et al. Molecular Aspects Concerning the Use of the SARS-CoV-2 Receptor Binding Domain as a Target for the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. mBio 2018, 9, e00211-18. [CrossRef]

182. Abatematteo, F.S.; Niso, M.; Contino, M.; Leopoldo, M.; Abate, C. Multi-Target Directed Ligands (MTDLs) Binding the σ(1) Receptor as Promising Therapeutics: State of the Art and Perspectives. Int. J. Mol. Sci. 2021, 22, 6359. [CrossRef]
185. Wang, N.; Shi, X.; Jiang, L.; Zhang, S.; Wang, D.; Tong, P.; Guo, D.; Fu, L.; Cui, Y.; Liu, X.; et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res.* 2013, 23, 986–993. [CrossRef] [PubMed]
186. Carvacho, I.; Piesche, M. RGD-binding integrins and TGF-β in SARS-CoV-2 infections—Novel targets to treat COVID-19 patients? *Clin. Transl. Immunol.* 2021, 10, e1240. [CrossRef] [PubMed]
187. Chen, Z.; Mi, L.; Xu, J.; Yu, J.; Wang, X.; Jiang, J.; Xing, J.; Shang, P.; Qian, A.; Li, Y.; et al. Function of HAb18G/CD147 in Invasion of Host Cells by Severe Acute Respiratory Syndrome Coronavirus. *J. Infect. Dis.* 2005, 191, 755–760. [CrossRef] [PubMed]
188. Wang, K.; Chen, W.; Zhang, Z.; Deng, Y.; Lian, J.; Du, P.; Wei, D.; Zhang, Y.; Sun, X.; Gong, L.; et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct. Target. Ther.* 2020, 5, 283. [CrossRef]
189. Kuzmicheva, G.A.; Jayanna, P.K.; Eroshkin, A.M.; Grishina, M.A.; Pereyaslavskaya, E.S.; Potemkin, V.A.; Petrenko, V.A. Mutations in fd phage major coat protein modulate affinity of the displayed peptide. *Protein Eng. Des. Sel.* 2009, 22, 631–639. [CrossRef]
190. Petrenko, V.A.; Smith, G.P.; Gong, X.; Quinn, T. A library of organic landscapes on filamentous phage. *Protein Eng. Des. Sel.* 1996, 9, 797–801. [CrossRef]
191. Petrenko, V.A.; Gillespie, J.W.; De Plano, L.M.; Shokhen, M.A. Phage-Displayed Mimotopes of SARS-CoV-2 Spike Protein Targeted to Authentic and Alternative Cellular Receptors. *Viruses* 2022, 14, 384. [CrossRef]
192. Thaper, D.; Prabha, V. Molecular mimicry: An explanation for autoimmune diseases and infertility. *Scand. J. Immunol.* 2018, 88, e12697. [CrossRef]
193. Wildner, G.; Diedrichs-Möhring, M. Molecular Mimicry and Uveitis. *Front. Immunol.* 2020, 11, 580636. [CrossRef]
194. Zhou, P.; Yang, X.-L.; Wang, X.-G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H.-R.; Zhu, Y.; Li, B.; Huang, C.-L.; et al. Addendum: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020, 588, E6. [CrossRef]
195. Li, Q.; Wu, J.; Nie, J.; Zhang, L.; Hao, H.; Liu, S.; Zhao, C.; Zhang, Q.; Liu, H.; Nie, L.; et al. The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell* 2020, 182, 1284–1294.e9. [CrossRef] [PubMed]
196. Kumar, S.; Sarma, P.; Kaur, H.; Prajapat, M.; Bhattacharyya, A.; Avti, P.; Sehkar, N.; Kaur, H.; Bansal, S.; Mahendiratta, S.; et al. Clinically relevant cell culture models and their significance in isolation, pathogenesis, vaccine development, repurposing and screening of new drugs for SARS-CoV-2: A systematic review. *Tissue Cell* 2021, 70, 101497. [CrossRef] [PubMed]
197. Chen, C.; Boorla, V.S.; Banerjee, D.; Chowdhury, R.; Cavener, V.S.; Nissly, R.H.; Gontu, A.; Boyle, N.R.; Vandegrift, K.; et al. Structure of MERS-CoV spike RBD and human ACE2. *Proc. Natl. Acad. Sci. USA* 2021, 118, e2106480118. [CrossRef] [PubMed]
198. Millet, J.K.; Jaimes, J.A.; Whittaker, G.R. Molecular diversity of coronavirus host cell entry receptors. *Nat. Microbiol.* 2020, 5, 149–153. [CrossRef]
199. Dias, R.; Torkamani, A. Artificial intelligence in clinical and genomic diagnostics. *Genome Med.* 2018, 10, 71–72. [CrossRef]
200. Wiens, J.; Shenoy, E.S. Machine Learning for Healthcare: On the Verge of a Major Shift in Healthcare Epidemiology. *Clin. Infect. Dis.* 2018, 66, 149–153. [CrossRef]
201. Shah, P.; Kendall, F.; Khozin, S.; Goosen, R.; Hu, J.; Laramie, J.; Ringel, M.; Schork, N.; et al. Robust prediction of HLA class II epitopes by deep motif deconvolution of immunopeptidomes. *Nat. Biotechnol.* 2019, 1284–1294.e9. [CrossRef] [PubMed]
202. Wallach, I.; Dzamba, M.; Heftets, A. AtomNet: A Deep Convolutional Neural Network for Bioactivity Prediction in Structure-based Drug Discovery. *arXiv* 2015, arXiv:1510.02855.
203. Ong, E.; Wong, M.U.; Huffman, A.; He, Y. COVID-19 Coronavirus Vaccine Design Using Reverse Vaccinology and Machine Learning. *Front. Immunol.* 2020, 11, 1581. [CrossRef]