Review Article

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COVID -19: From the Molecular Mechanisms to Treatment

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The 2019 novel coronavirus (SARS-CoV-2) causes severe pneumonia called COVID-19 and leads to severe acute respiratory syndrome with a high mortality rate. The SARS-CoV-2 virus in the human body leads to jumpstarting immune reactions and multi-organ inflammation, which has poorer outcomes in the presence of predisposing conditions, including hypertension, dyslipidemia, dysglycemia, abnormal adiposity, and even endothelial dysfunction via biomolecular mechanisms. In addition, leucopenia, hypoxemia, and high levels of both cytokines and chemokines in the acute phase of this disease, as well as some abnormalities in chest CT images, were reported in most patients. The spike protein in SARS-CoV-2, the primary cell surface protein, helps the virus anchor and enter the human host cells. Additionally, new mutations have mainly happened for spike protein, which has promoted the infection's transmissibility and severity, which may influence manufactured vaccines' efficacy. The exact mechanisms of the pathogenesis, besides molecular aspects of COVID-19 related to the disease stages, are not well known. The altered molecular functions in the case of immune responses, including T CD4+, CD8+, and NK cells, besides the overactivity in other components and outstanding factors in cytokines like interleukin-2, were involved in severe cases of SARS-CoV-2. Accordingly, it is highly needed to identify the SARS-CoV-2 bio-molecular characteristics to help identify the pathogenesis of COVID-19. This study aimed to investigate the bio-molecular aspects of SARS-CoV-2 infection, focusing on novel SARS-CoV-2 variants and their effects on vaccine efficacy.

Key words: COVID -19; Molecular Mechanisms; SARS-CoV-2; Treatment

The coronavirus disease 2019 (COVID-19) spread worldwide, and after that, WHO announced COVID-19 as a Public Health Emergency International Concern (PHEIC) (1). COVID-19 management and control heavily rely on health capacity worldwide (2). Of note, it is demonstrated that COVID-19 spreads faster than other coronaviruses in the human population (3).

SARS-CoV-2 belongs to the Coronaviridae family, including other respiratory virus members such as SARS and MERS. The genome of nCoV-19 is described in detail as single-stranded (positive-sense) RNA surrounded by a cohesive matrix protein capsid. The typical genome of the virus has at least six ORFs that can encode functional and structural proteins. Moreover, the SARS-CoV-2 genome encodes structural proteins, including S, M, N, and E, and non-structural proteins (4). Nevertheless, no precise mechanisms are identified for the rapid spread of this virus throughout the world (5). There are different severity levels regarding clinical manifestations in patients with COVID-19 (6). There are three stages for COVID-19 disease related to the critical therapeutic strategies and the involvement of different interesting molecular pathways

during viral infection parallel to the involvement of various body organs, as shown in Figure 1.

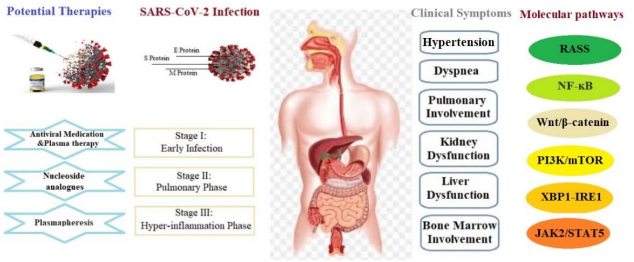

Figure 1. Clinical symptoms and stages of COVID-19 disease, related molecular pathways and therapeutic strategies during viral infection parallel to involvement of various organs of the body

Moreover, multiple implications of personalized medicine on the severity of the disease and novel variants of SARS-CoV-2 have been demonstrated. Due to the nature of RNA viruses, there are more possibilities for new mutations (1). In this review, we attempted to investigate biomolecular mechanisms, current treatment options, and novel mutations that help distribute SARS-CoV-2-induced infection.

Immuno-pathophysiology of COVID-19

COVID19 binds to ACE2 receptors on human lung epithelial cells via its spike protein. Accordingly, it is primed by type II transmembrane serine protease (TMPRSS2). Both dysregulation and aberrant activity of CD8+ T cells occur in severe cases of COVID-19. In extreme cases, high levels of IL-6 and IL-8 during the treatment were associated with low lymphocyte counts. Many new clinical trials were performed on HrsACE2, which reported promising results.

The viral infection outcomes are respiratory disorders and cytokine storm syndrome. Cytokine storm syndrome mainly occurs due to un-regulated inflammatory immune responses and the up-regulation of the expression of inflammatory cytokines (7). Cytokine storm causes inflammatory-induced lung injury, which consequently induces pneumonia, lung failure, acute respiratory distress syndrome (ARDs), shock, and even death among the infected individuals depending on their genetic backgrounds. The risk of death is also high among older adults and people with high blood pressure, uncontrolled diabetes, immunodeficiency, and cardiovascular disease (7).

Some previous studies have indicated that individuals infected by the SARS-CoV-2 virus showed neutrophilia and the expression of cytokines and chemokines such as TNF-α, CCL-2, CXCL-10, and MIP-1α as well as acute phase proteins, besides the upregulation of CRP in their blood (8). The SARS-CoV-2 virus targets type 2 lung alveoli cells, which express the ACE-2 receptor at high levels, and then enter the cells through binding to the ACE-2 receptor. Correspondingly, the ACE-2 receptor is expressed at low levels on the surface of macrophages/ monocytes (9). Up to now, the mechanisms involved in the SARS-CoV-2 virus infecting immune cells has remained unknown. After the entrance of this virus into the cells, the sense RNA of the virus is released and replicates. Following the replication of the virus, new viruses are released from the cells and the infected cells and then produce inflammatory cytokines and type 1 interferon (10). The neutrophils, macrophages, and monocytes are then recruited to the lungs in response to inflammatory cytokines that cause inflammation-induced lung injury (11). Besides altered interferon production, the virus is recognized by TLR-3/7 or RIG-1 and MDA5, followed by its recognition and downstream signaling pathways. However, this virus employs various mechanisms to escape from the immune responses in the human body. In this regard, the probable mechanisms interfere with the production of type 1 interferon, ubiquitination of RNA sensors and intermediate molecules such as TRAFT3 and TRAF6, the inhibition of nuclear translocation factor (IRF-3), down-regulation of MHC-I expression by infected cells, and down-regulation of MHC-expression by antigen-presenting cells (12).

Novel global variants of SARS-CoV-2 strains

Molecular aspects, including genetic coronavirus variations, have revealed some novel facts about SARS-CoV-2, including the deletions and hotspots in ORF8 and

milder clinical symptoms with less proinflammatory cytokines in patients infected with COVID-19 (6). According to novel studies, different mutation patterns depend on mutated enzymes like RdRP (RNA dependent). These are considered the most important enzymes for viral phenotype and behavior for discovery of the best treatment procedure and drug-targeting enzymes. Different SARS-CoV-2 mutation hot spots in geographic areas also affect mortality rate (13). By tracing and monitoring the involved genetic annotation, 12 genomic deletion sites other than the CONFIRMED reports in ORF8, spike, ORFa protein, ORF1ab polyprotein, ORF10, and 3'-UTR have been found (14).

SARS-CoV-2 is rapidly transmitted in susceptible individuals. Furthermore, novel variants with different transmission abilities have begun disseminate globally in different countries. New mutations of SARS-CoV-2 mainly occur in the spike protein (15). The spike protein hides the virus from the immune system and is the first target for producing antibodies against the virus's processes of recognizing and neutralizing the pathogen. RNA viruses, such as coronaviruses, constantly undergo different mutations. Although some of these mutations have no significant effect on viral characteristics, others can increase their selectivity advantages, such as transmissibility (16, 17). Therefore, these mutations need to be monitored closely. Recently, some studies have reported the emergence of SARS-CoV-2 variants in different countries such as the UK(20i/501Y.V1/B.1.1.7), Brazil(P.1/20j/501y.v3/b.1.1.248), and South Africa (20H/501Y.V2/B.1.351) (18). Mutations that can change the amino acids and building blocks in viral proteins also alter their characteristics. Most prevalent mutations, including the substitution of amino-acid D614G, are in the spike protein of SARS-CoV-2 and can modulate the viral entrance to the host cells (19). This substitution is known as a linkage disequilibrium with the mutation of ORF1bp314L. The potentials of spike D614G for increasing the pathogenesis of virus-induced disease has also been confirmed (20). These findings aligned with cell fusion data using the spike protein and the expressed ACE2 in various cells. One of the novel SARS-CoV-2 variants, B.1.617, carried two identified mutations, one in the position of 454 in spike protein and the second in the position of 484, but has not been recognized as a double mutation. B.1.617 includes various positions of spike protein which is necessary for anchoring to the ACE2 receptor on the pulmonary cell surface and other human cells. Eight mutations in B.1.617 have been found in the middle point of immature spike protein; novel mutations increase transmissibility and the incidence of severe COVID-19 disease (21).

Molecular events in viral-infected cells

The spike protein is a crucial identification factor for virus linkage and entrance to the host cells, which is present on the outer surface of SARS-CoV-2 (22, 23). The master chaperone protein responding to these alterations is glucose regulating protein 78 (GRP78) or binding immunoglobulin protein (BiP) (24-27). Accordingly, GRP78 located in the endoplasmic reticulum lumen (ER) inactivates enzymes involved in cell differentiation and cell death. Moreover, these enzymes trigger the activity of transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and inositol-requiring enzyme 1 (IRE1) (23). In addition, it is demonstrated that GRP78 releases ATF6, PERK, and IRE1 (23, 28). GRP78 overexpression is also induced upon cell stress, which consequently increases the possibility of GRP78 leaving ER and translocating to the cell membrane. Of note, GRP78 is responsive to viral identification via its substrate-binding domain (SBD) and may mediate the entry of the virus into the cell (23). A cyclic peptide called Pep42 linked to GRP78 has been identified outside infected cells (29). Therefore, SARS-CoV-2 spike identification may occur by the cell-surface GRP78 upon cell stress (30). This information seems promising for the detection of virus-infected cells.

The emergence of the Middle East respiratory syndrome (MERS)-CoV and severe acute respiratory

syndrome coronavirus (SARS-CoV) impose the risk of cross-species transmission events starting to outbreak among human populations. In this regard, viruses encoding the SHC014 spike in a wild-type backbone can use various types of the SARS receptors of human angiotensin-converting enzyme II (ACE2) with attachment ability which can efficiently attach to the primary human airway epithelial cells. Additionally, previous in-vivo studies demonstrated the development of the chimeric virus in the mice respiratory system with considerable pathogenesis.

Molecular methods of SARS-CoV-2 detection

Various diagnostic methods such as serology tests, cell culture, nucleic acid amplification-based methods, CRISPR-based diagnostics, and direct immunofluorescence (DIF) staining of viral-specific antigens are employed to detect influenza viruses (31, 32). Moreover, the routine diagnostic tests performed for virus-infected patients consist of several steps, including isolating viral particles from cell culture and analyzing the obtained samples using serological tests (33). Accordingly, serological tests are the first to detect respiratory virus infections (34). From 1970 to the 1980s, to isolate respiratory viruses, three or four cell lines were used besides the embryonated hens' eggs (35). Moreover, various serology tests based on the fold changes in the antibody titers of patients' sera, including the complement fixation test (CFT), hemagglutination inhibition test (HAI), and immune enzyme assay(EIA), were utilized in this regard (36). By introducing shell vial culture and the specified monoclonal antibodies against viral antigens, viral-related antigens were identified during the first 1- 2 days in cell culture (sooner than the culture tube lasting 8-10 days) (37). Developing direct immunofluorescence (DIF) staining of nasopharyngeal specimens in the 1990s has facilitated the detection of viruses. However, the low sensitivity of DIF has substantially restricted its application. Molecular detection methods based on nucleic acid-based amplification such as NATs, RT PCR, Real-time PCR, Nested PCR, multiplex PCR, and microarrays are considered the most novel diagnostic methods in clinical labs. Molecular diagnostic tests are more sensitive, accurate, and fast than DFA and culture (38). It is noteworthy that detection antibodies against viral antigens are susceptible and specific diagnostic methods. Still, changes in antibody titers in patient sera can be distinguished as late as about ten days from the infection (39). Molecular detection approaches based on PCR help diagnose early-stage viral diseases (40). Serological tests can be applied for SARS-CoV-2 virus detection after identifying COVID-19-associated antigens and providing specific antibodies against the antigens soon (41).

Molecular approaches for fast recognition of COVID-19

The advances in molecular techniques have led to fast recognition of the novel coronavirus. The first step to identification of the SARS-CoV-2 virus in samples obtained from patients is to isolate the virus and obtain its nucleic acid (42, 43). Accordingly, recent studies have indicated several diagnostic approaches such as RT-LAMP (reverse transcription loop-mediated isothermal amplification), RT-iiPCR (RT-insulated isothermal PCR), and a one-step rRT-PCR assay specific TaqMan probes-based. The sensitivity of RT-LAMP is similar to q-RT-PCR, and its advantages include specificity and convenience (44). Similarly, one-step rRT-PCR and RT-iiPCR assays have demonstrated high diagnostic specificity and sensitivity for the coronavirus (45, 46). Since the whole genome sequence of the SARS-CoV-2 virus has been completely identified, suspected cases can be detected via pan-coronavirus PCR test. Additionally, RT-PCR has been applied to detect samples collected from respiratory tracts of individuals suspected of COVID-19-suspected cases (38). Optimized RT PCR methods like q-RT-PCR were also used to detect the MERS-Corona virus envelope gene and the open reading frame 1a/1b genes (47). On the other hand, real-time RT-PCR was employed to detect COVID-19 by detecting the E gene, RNA-dependent RNA polymerase gene, and N gene of this virus. Several other molecular

methods have also been introduced, like RT-LAMP (a technique based on RNA amplification), which detects the N gene and ORF1a of the MERS-Corona Virus (48). Moreover, one-pot reverse transcription loop-mediated isothermal amplification (optimized RT-LAMP) and RT-LAMP-VF(deformation of RT-LAMP) are specifically employed to identify the MERS-Corona virus N gene faster and more efficiently than other methods (49). MALDI-TOF Mass Spectrometry is another molecular-based technique that can precisely recognize identified human coronaviruses and give phylogenetic data regarding unidentified ones (50). The multiple-target sensor is another approach utilized to amplify the target and facilitate the pathogen diagnosis in clinical specimens (51).

In COVID-19 detection, **c**hest CT has been estimated to be sensitive in about 56-98% of cases. Therefore, it could be used to confirm false negative results obtained from q-RT-PCR, especially at the early stages of the disease (52). In the early phases, the lesion pattern was observed to be similar to GGO in the middle areas of the lung lobes. Still, by progressing the disease, the lesion pattern changes to a crazy-paving pattern in several lobes of the lungs. Therefore, besides molecular-based tests and clinical manifestations, CT can facilitate accurate detection in individuals infected with Coronavirus (53).

Pharmacotherapy and SARS-CoV-2 virus

Potential therapeutic targets for SARS-CoV-2 virus

The SARS-CoV-2 virus binds to ACE2 receptors on human epithelial cells via its spike protein which is primed by the type II transmembrane serine protease (TMPRSS2). Several drugs (such as chloroquine, hydroxychloroquine, and camostat mesylate) are demonstrated to work by virus attachment inhibition through both ACE2 and TMPRSS2 targets (54-56).

Regarding pharmacokinetic vision, the virus enters the host cell by endocytosis and then forms endo-lysosomes to facilitate viral uncoating and fusion. Viral proteins, including cathepsin L and cathepsin B at acidic endo-lysosomal pH, undergo enzymatic alterations by lysosome proteases. Then the fusion and release of viral RNA into the human cytoplasm occur. The endo-lysosomal acidic pH and the proper function of lysosomal enzymes play essential roles in viral infectivity. Therefore, it has been hypothesized that some medications (such as azithromycin, glycoprotein antibiotics, triazole antifungals, chloroquine, and remdesivir) may affect the function of lysosomes, thereby decreasing viral spread (55). As well, the inhibition of clathrin-mediated endocytosis through the inhibition of the numb-associated kinase (NAK) family (AAK1 and GAK) has been suggested as another potential target for the SARS-CoV-2 virus (56). The virus uses host cell structures for genome replication and protein synthesis processes by releasing RNA into the cytoplasm. Many antiviral medications inhibiting RNA-dependent RNA polymerase (RdRp) and viral protease also interfere with the virus replication (56,57). In cases with severe infection, it has been observed that the host's immune system exhibits an uncontrolled inflammatory response, resulting in the development of many complications, such as ARDS. Several drugs, including glucocorticoids, IVIG, interferon products, and inhibitors of IL6, IL2, and JAK, act through modulation of such response (58).

Another mechanism for chloroquine has been previously proposed in a recent modeling study. According to this study, ORF10, ORF1ab, and ORF3a (non-structural coronavirus proteins) attack the heme and separate iron from porphyrin. Subsequently, other surface proteins (ORF8 and E2 glycoprotein) bind to the porphyrin and form stable complexes. Altogether, these mechanisms lead to the accumulation of iron and prevent its usage in anabolic pathways. This study has stated that chloroquine phosphate competes with the porphyrin binding to viral proteins. Therefore, it can inhibit the virus's attack on the heme and prevent the formation of porphyrin complexes (57). Pharmacotherapy and mechanisms of drugs by focusing on targeting the host cell and viral structure and considering the method of analysis are tabulated in Table 1.

SARS-CoV-2 Virus's Therapeutic Approaches and Clinical Trials

A Survey of the available SARS-based immune-therapeutic and prophylactic modalities revealed poor progress of both monoclonal antibody and vaccine approaches to neutralize the virus and protect people against SARS-CoV-2 using the new spike protein. Based on these findings, a study synthetically re-derived an infectious full-length SHC014 recombinant virus and confirmed the solid viral development under *in vitro* and *in vivo* conditions. Their study implied a potential risk of SARS-CoV re-emergence from viruses currently circulating in bat populations (58).

Based on the clinical trial categories, 23 different pharmacological classes are currently understudied for the treatment of COVID-19. The drugs tested against COVID-19 range from anti-malaria medications to approved flu drugs. All these categories are summarized in Table 2. Antiviral drugs have received much attention, with 33 records in clinical trials, and they have been incorporated into treatment protocols in different countries. Up to April 2020, 220 worldwide clinical trials had been registered to treat coronavirus disease. The drugs included in these studies, with their pharmacokinetic properties, are listed in Table 3. Researchers are applying immune-base therapeutic interventions, including IL-6 antagonists (ChiCTR2000029765), and some new molecular procedures, such as mesenchymal stem cells (NCT04252118), which have shown promising results. The severity of the COVID-19 infectious disease depended more on the host-related response factors, including age, immune system strength, lymphocytopenia, and cytokine storm.

The efficacy of vaccines may be affected by new mutations

Since the spike protein in the SARS-CoV-2 envelope has been used as the primary target for designing vaccines, new challenges were faced by scientists and vaccine development companies (59). Besides considering molecular aspects in coronavirus, including genetic variations, novel information about SARS-CoV-2 has emerged, including the deletions in the hotspot of ORF8 that resulted in milder clinical symptoms with less proinflammatory cytokines in patients with COVID-19. By tracing and monitoring the involved genetic annotations, 12 genomic deletion sites have been found other than the confirmed reports in ORF8, spike, ORFa protein, ORF1ab polyprotein, ORF10, and 3'-UTR. These findings align with cell fusion data using the spike protein and the expressed ACE2 in various cells. One of the novel SARS-CoV-2 variants, B.1.617, carried two identified mutations, one in the position of 454 in spike protein and the other in the position of 484, but they have not been named as double mutations. New studies have shown that the nCoV-19 vaccine may act against the B.1.351 variant by neutralizing antibody responses to the COVID-19 infection (21). Researchers have also reported that the B.1.617.1 variant may be 6.8-fold more resistant than other variants in individuals vaccinated against COVID-19 with Moderna and Pfizer vaccines (21).

CONCLUSION

In conclusion, in the current coronavirus pandemic, amongst all actions, the increased surveillance and activities aiming at the identification of suspected individuals can be valuable for future management of the disease. Moreover, through patient transference and separation, fast diagnosis, detection, and case monitoring of potential contacts, as well as designing the new vaccines and new targets for specific drugs, the COVID-19 infection can be controlled. The utilization of such a vast technological and operational collection of intercessions depends on the decisions made by public health and laboratory bases and resources in each country. An effective disinfection procedure should include a pre-cleaning step to remove these organic materials. Pharmacotherapy and consideration of the molecular mechanisms of drugs by focusing on targeting the host cells and viral structure besides using novel platforms, for instance, nucleic acid-based vaccines, are still valuable.

Table 1. Pharmacotherapy and mechanisms of drugs in focus on targeting the host cell and viral structure considering the method of analysis

Medication	Target in human cells	Targets in viral structure	Evidence/Method
Chloroquine	ACE2		Cell-based assay (11)
	lysosome pH increment		Cell-based assay (11) immunofluorescence analysis and confocal microscopy (12)
	cathepsin L		Hypothesis (4)
		papain-like protease (PL-PRO)	Docking (13)
		ORF10 ORF1ab ORF3a	modeling and docking (10)
		ORF8 Glycoprotein E2	modeling and docking (10)
Hydroxychloroquine	ACE2		Cell-based assay (12)
	lysosome pH increment		Cell-based assay, immunofluorescence analysis and confocal microscopy (12)
	cathepsin L		Hypothesis (4)
	IL6		Hypothesis (14)
Camostat mesylate	TMPRSS2		Cell-based assay (3)
Remdesivir		RdRp (NTP binding motif)	Docking and molecular dynamics simulations (6, 15, 16) Cell-based assay (11)
	A2a receptor (Lysosome dysfunction)		Hypothesis (4)
Lopinavir/ritonavir		3C-like proteinase (3CL-PRO)	Docking and molecular dynamics simulations (7, 16)
Atazanavir		Viral protease	Virtual screening (17) Virtual screening (17)
Oseltamivir		3CL-PRO	Docking (16)
Indinavir		3CL-PRO	Docking (6) Virtual screening (17)
Ribavirin		RdRp	Hypothesis (2, 18)
Nelfinavir		3CL-PRO	Docking (16)
Umifenovir (arbidol)	ACE2	Spike protein	Hypothesis (2, 18) Cell-based assay (19)
Favipiravir		RdRp	Hypothesis (20, 21)
Darunavir		Viral protease Papain-like protease 3CL-PRO	Docking (22) Multi-task deep modeling (23)
Saquinavir		main protease	Virtual screening (24)
Beclabuvir		main protease	Virtual screening (24)
Cobicistat		3CL-PRO	Virtual screening (17)
Grazoprevir		PL-PRO	Docking (25)
Telaprevir		PL-PRO	Docking (25)
Boceprevir		PL-PRO	Docking (25)
Carfilzomib		3CL-PRO	Docking and molecular dynamics simulations (7)
Eravacycline		3CL-PRO	Docking and molecular dynamics simulations (7)
Valrubicin		3CL-PRO	Docking and molecular dynamics simulations (7)
Elbasvir		3CL-PRO	Docking and molecular dynamics simulations (7)
Azithromycin	Lysosome pH increment		Hypothesis (4)
Triazole antifungals (posaconazole and itraconazole)		Viral helicase	Hypothesis (4)

	protein NPC1		
Glycopeptide antibiotics (Teicoplanin and dalbavancin)	cathepsin L		Cell-based assay (26)
Streptomycin		3CL-PRO	Docking and molecular dynamics simulations (7)
Tocilizumab	IL6		Hypothesis (20, 21)
Baricitinib	Numb-associated kinase (AAK1 /GAK)		Hypothesis (5, 27)
Sorafenib	JAK-STAT signaling stimulator of interferon genes protein (STING)		Cell-based assay (28)
Ciclesonide		amino acid (A25V) in nonstructural protein 15	Cell-based assay (29)
Thalidomide	TNF-alpha IL-6 IL-1β		Hypothesis (30)
Lithium	glycogen synthase kinase 3 beta (GSK-3β)		Hypothesis (31)
Methylprednisolone	Cytokine storm		Hypothesis (20, 21)
Interferon products	IFN signaling pathway		Hypothesis (32)
Nitazoxanide	melanoma differentiation-associated gene 5 (MDA5) mitochondrial antiviral-signaling protein (MAVS) IFN- β		Hypothesis (14)
Mycophenolic acid		PL-PRO	Docking (25)
Cvl218	poly-ADP-ribose polymerase 1 (PARP1)		Docking and Cell-based assay (19)

Table 2. Summarized categories of drugs being tested to help fight COVID-19 differ from anti-malaria treatments to approved flu drugs.

COVID-19 Drug Interventions by Category
Analgesics
• Anti-Arrhythmia Agents
• Anti-Infective Agents
• Anti-Inflammatory Agents
• Anticoagulants
Antiemetics
Antihypertensive Agents
Antineoplastic Agents
• Antirheumatic Agents
• Bone Density Conservation Agents
• Central Nervous System Depressants
• Channel Blockers
• Dermatologic Agents
• Gastrointestinal Agents
• Lipid Regulating Agents
• Micronutrients
• Neuroprotective Agents
• Reproductive Control Agents
• Renal agents
• Respiratory System Agents
• Urological Agents
• Vasoconstrictor Agents
• Vasodilator Agents

Table 3. Lists of drugs included in these studies with their pharmacokinetic properties

No.	NCT Number	Pharmacological Classification	Intervention	Pharmacokinetic
1	NCT04288713	Long acting humanized monoclonal antibody targeted against complement C5	•Drug: Eculizumab	• Peak serum concentration (at week 26): 194 mcg/mL • Trough concentration (at week 26): 97 mcg/mL • Vd: 7.7 L • Half-Life: 8-15 days • Half-Life following plasma exchange: 1.26 hours • Clearance: 22 mL/hr/70 kg • Clearance following plasma exhange: 3660 mL/hr
2	NCT04276688 NCT04255017 NCT04295551 NCT04291729 NCT04261907 NCT04286503 NCT043211993 NCT04307693 NCT04321174	HIV protease inhibitor	•Drug: Lopinavir/ritonavir	**Lopinavir** • Peak Plasma Time: 4 hr • Peak Plasma Concentration: (800 mg qDay x 4 wk): 11.8±3.7 mcg/mL • Half-life: 5-6 hr • Protein Bound: 98-99% • Metabolism: CYP3A4 which is inhibited by ritonavir • Excretion: Feces (83%); urine (10%) **Ritonavir** • Absorption: variable, with or without food • Vd: 0.16-0.66 L/kg (high concentrations in serum & lymph nodes) • Protein Bound: 98-99% • Metabolism: Hepatic; five metabolites, low concentration of an active metabolite achieved in plasma (oxidative) • Half-life: 3-5 hr • Peak plasma time: 2 hr (oral solution) • Excretion: Urine (11%); feces (86%)
3	NCT04276688	Guanosine Analog	•Drug: Ribavirin	• Absorption • Absorption (inhalation): Systemic maximal absorption occurs with use of aerosol generator via endotracheal tube; highest concentrations may occur in respiratory tract and erythrocytes • Peak plasma time: 3 hr (multiple doses; capsule at end of inhalation period) • Bioavailability: 64% (PO) • Distribution • Significantly prolonged in erythrocyte (16-40 days), which may use as marker for intracellular metabolism • Vd: 2825 L • Protein bound: None (PO) • Metabolism • Hepatically and intracellularly (forms active metabolites); may be necessary for drug action • Elimination • Half-life: 24 hr in healthy adults (capsule); 44 hr (chronic hepatitis C infection; increases to ~298 hr at steady state) • Excretion: Urine (61%); feces (12%)
4	NCT04276688	Immunomodulator	•Drug: Interferon Beta-1B	• Peak plasma time: 1-8 hr • Concentration: 40 IU/mL • Half-Life: 8 min - 4.3 hr • Onset: 6-12 hr • Bioavailability: 50% • Vd: 0.25-2.88 L/kg • Clearance: 9.4-28.9 mL/min
5	NCT04293887		•Drug: Recombinant Human Interferon α1β	• Half-Life: 2-3 hr (IM/SC); 2 hr (IV) • Peak Plasma Time: 3-12 hr (IM/SC); 30 min (IV) • Pharmacogenomics • Polymorphic cytokine genes (encoding IL-10, a Th2 cytokine); Th2 responses are associated with production of large amounts of antibodies

				• Patients with chronic hepatitis C are 5 times more likely to have a favorable response to interferon alfa if they carried the IL-10 genetic polymorphism that results in low expression of IL-10 than if they did not
6	NCT04273321 NCT04323592 NCT04263402 NCT04273581 NCT04244591	Glucocorticoids and Anti inflammation	• Drug: Methylprednisolone (Methylprednisolone Acetate/ Hemisuccinate)	• Absorption • Onset: 1-2 hr (PO); 4-8 days (IM); 1 week (intra-articular) • Duration: 30-36 hr (PO); 1-4 weeks (IM) • Peak plasma time: 31 min (IV) • Distribution • Vd: 0.7-1.5 L/kg • Metabolism • Extensively metabolized in liver • Elimination • Half-life: 3-3.5 hr • Dialyzable: Hemodialysis, slightly • Total body clearance: 16-21 L/hr • Excretion: Urine (mainly, as metabolites), feces (minimally)
7	 NCT04279197	Mucolytic	•Drug: N-acetylcysteine	• Absorption • Onset: 5-10 min • Peak plasma time: 1-2 hr • Distribution • Duration: Variable (~1 hr) • Protein bound: 80% • Metabolism • Metabolized in liver • Elimination • Excretion: Urine (primarily)
8	 NCT04261426	Immunoglobulin	•Drug: Intravenous Immunoglobulin	• Not defined
9	 NCT04320277	Janus kinase inhibitor	•Drug:Baricitinab	• Bioavailability: 79% • Protein binding: 50% • Metabolism: CYP3A4 (<10%) • Elimination half-life: 12.5 hours • Excretion: 75% urine, 20% faeces
10	NCT04264533 NCT04323514 NCT03680247 NCT04323228	Vitamins and Trace elements	• Drug: Vit C (Ascorbic Acid) •Drug: Selenium •Drug: Vitamin A	• Distribution: Large • Metabolism: Liver • Absorption: Rapidly absorbed • Excretion: Urine • Excretion: Urine, feces, lungs, skin • Serum concentration: 300-700 ng/mL (adults); 200-500 ng/mL (infants) • Peak plasma time: 4-5 hr (oil solution); 304 hr (water-miscible) • Protein Bound: Retinol binding protein • Distribution: Mainly stored in liver as retinyl palmitate • Metabolism: hepatic glucuronidation, decarboxylation • Metabolites: retinoic acid, retinal • Excretion: Urine and feces (via bile)
			•Drug: Vitamin E	• Absorption: Reduced in patients with history of malabsorption; water preparations better absorbed than oil preparations • Distribution: All body tissues especially adipose tissues where it is stored • Metabolism: Liver • Excretion: Feces
11	 NCT04326725		•Drug: Zinc	• Half-life: 11 days following cessation of therapy (inhibition of copper uptake) • Absorption: pH dependent (enhanced at pH<3); impaired by food • Excretion: Feces (primarily)

12	NCT04273763	Mucolytic , secretolytic and secretomotoric	•Drug: Bromhexine Hydrochloride	• Peak plasma time: 1 h • Distribution: Large • Metabolism: Liver • Absorption: Rapidly absorbed • Excretion: Urine and bile. • Bioavailability: 75-80% • Elimination half-life: 12 hr •
	NCT04273763 NCT04255017 NCT04254874 NCT04286503 NCT04260594	Membrane Fusion Inhibitor	•Drug: Umifenovir	• Bioavailability: 40% • Metabolism: Hepatic • Elimination half-life: 17-21 hours • Excretion: 40% excrete as unchanged umifenovir in feces (38.9%) and urine (0.12%)
	NCT04273763	RNA dependent RNA polymerase inhibitor	•Drug: Favipiravir	• Concentration-dependent aldehyde oxidase inhibition
13	NCT04273529 NCT04273581	Immunomodulator and anti-inflammatory ,tumor necrosis factor alpha Suppressor, angiogenes inhibitor	•Drug: Thalidomide	• Bioavailability: 90% • Protein bound: 55-66% • Peak plasma time: 3-6 hr • Half-life: 5-7 hr • Peak plasma concentration: 1.15-3.2 mcg/mL • Vd: 122 L • Metabolism: Liver • Clearance: 1.15 mL/min • Excretion: Urine
14	NCT04280588	Immunomodulator (sphingosine-1-phosphate receptor modulator)	•Drug: Fingolimod	• Absorption • Bioavailability: 93% • Plasma Time: 12-16 hr • Steady-State: Time to steady state is 1-2 months following daily dosing; steady-state levels approximately 10-fold greater than initial dose • Distribution • 86% distributed in RBCs • Protein Bound: >99% • Vd: 1200 L • Metabolism • Primarily by CYP4F2, minor substrate of CYP2D6, 2E1, 3A4, and 4F12 • Elimination • Half-life: 6-9 days • Clearance: 6.3 L/hr • Excretion: Feces (<2.5%), urine (81% as inactive metabolites)
15	NCT04275414	Antineoplastic agent	•Drug: Bevacizumab	• Mechanism of Action • Recombinant humanized monoclonal antibody to VEGF; blocks the angiogenic molecule VEGF thereby inhibiting tumor angiogenesis, starving tumor of blood and nutrients • Absorption • Steady-state concentration is 84 days • Accumulation ratio: 2.8 (following 10 mg/kg dose) • Distribution • Vd: 2.9 L (mean); 3.2 L (males); 2.7 L (females) • Elimination • Clearance: 0.23 L/day (mean); 0.26 L/day (males); 0.21 L/day (women) • Half-life: 20 days •
16	NCT04261270 NCT04254874	Neuraminidase inhibitor	•Drug: Oseltamivir	• Absorption • Bioavailability: 75% • Peak plasma time: 2.5-6 hr • Distribution • Protein bound: 3% (oseltamivir carboxylase); 42% (oseltamivir) • Vd: 23-26 L • Elimination • Half-life: 1-3hr (oseltamivir); 6-10 hr (oseltamivir carboxylate)

				• Excretion: Feces; urine (>90% as oseltamivir carboxylate) •
17	NCT04261517 NCT04323631 NCT04307693 NCT04315896 NCT04318444 NCT04318015 NCT04326725 NCT04303507 NCT04321278 NCT04325893 NCT04322123 NCT04323345 NCT04303299 NCT04321993 NCT04322396 NCT04321616 NCT04261517 NCT04315948 NCT02735707 NCT04308668 NCT04304053 NCT04320277 NCT04286503 NCT04324463 NCT04323527 NCT04322396 NCT04322396	Anti-malaria and antirheumatic,anti-inflammatory and immunomodulatory effects	•Drug: Hydroxychloroquine •Drug:Chloroquine diphosphate/ Chloroquine	• Absorption • Peak plasma concentration: 129.6 ng/mL (single 200-mg dose) • Peak plasma time: 3.26 hr (single 200-mg dose); 3-4 hr (chronic PO administration) • Metabolism • Metabolites: Desethylhydroxychloroquine, desethylchloroquine • Elimination • Half-life: 40-50 days
18	NCT04252274	Protease Inhibitor /selectively inhibits cleavage of Gag-Pol polyprotein precursors	•Drug: Darunavir	• Absorption • Peak plasma time: 4-4.5 hr (darunavir); 4-5 hr (cobicistat) • Trough concentration (darunavir): 1875 ng/mL • AUC (darunavir): 100,152 ng·hr/mL High-fat meal increases absorption; darunavir/cobicistat should be taken with food • Distribution • Protein bound: 95% (darunavir); 97-98% (cobicistat) • Metabolism • Darunavir: Primarily undergoes oxidative metabolism; extensively metabolized by CYP enzymes, primarily by CYP3A • Cobicistat: Metabolized by CYP3A and to a minor extent by CYP2D6 enzymes and does not undergo glucuronidation • Elimination • Half-life: 7 hr (darunavir); 4 hr (cobicistat) • Excretion • Darunavir: 79.5% feces (41.2% unchanged); 13.9% urine (7.7% unchanged) • Cobicistat: 86.2% feces; 8.2% urine
		Cytochrome **P450** (CYP) inhibitor	•Drug: Cobicistat	• Absorption • Peak plasma time: 4-5 hr (cobicistat) • Distribution • Protein bound: 97-98% (cobicistat) • Metabolism • Cobicistat: Metabolized by CYP3A and to a minor extent by CYP2D6 enzymes and does not undergo glucuronidation • Elimination • Half-life: 4 hr (cobicistat) • Excretion • Cobicistat: 86.2% feces; 8.2% urine
19	NCT04325633	**Anti-inflammatory**	•Drug: Naproxen	• Absorption • Bioavailability: 95%

				• Onset: 30-60 min • Duration: < 12 hr • Peak serum time: 1-4 hr (tablets); 2-12 hr (delayed release empty stomach); 4-24 hr (delayed relase with food) • Peak plasma concentration: 62-96 mcg/mL • Distribution • Protein bound: <99% • Vd: 0.16 L/kg • Metabolism • Metabolized in liver via conjugation • Metabolites: 6-Desmethylnaproxen, glucuronide conjugates • Enzymes inhibited: COX-1, COX-2 • Elimination • Half-life: 12-17 hr • Dialyzable: No value • Clearance: 0.13 mL/min/kg • Excretion: Urine (95%), feces (<3%)
20	NCT04312009 NCT04311177	**Angiotensin II receptor blocker**	•Dru: Losartan	• Absorption • Bioavailability: 25% • Onset: 6 hr • Duration: 24 hr • Peak plasma time: 1-1.5 hr • Distribution • Protein bound: Losartan, 98.7%; E-3174, 99.8% • Vd: Losartan, 34 L; E-3174, 12 L • Metabolism • Metabolized by hepatic P450 enzyme CYP2C9 • Metabolites: 5-Carboxylic acid (E-3174) (active metabolite; 40 times as potent as losartan in angiotensin II-blocking activity) • Elimination • Half-life: Losartan, 1.5-2 hr; E-3174, 6-9 hr; increased in end-stage renal failure or CHF • Dialyzable: HD, no; PD, no • Renal clearance: Losartan, 43-75 mL/min; E-3174, 18-25 mL/min • Total plasma clearance: Losartan, 600 mL/min; E-3174, 50 mL/min • Excretion: Urine (4%)
21	NCT04324463 NCT04322396	Macrolides (Binds to 50S ribosomal subunit of susceptible microorganisms and blocks dissociation of peptidyl tRNA from ribosomes, causing RNA-dependent protein synthesis to arrest)	•Drug: Azithromycin	• Absorption • Absolute bioavailability: 38% (250-mg capsules) • Peak plasma concentration • Oral (3-day regimen): 0.44 mcg/mL (Day 1); 0.54 mcg/mL (Day 3) • Oral (5-day regimen): 0.43 mcg/mL (Day 1); 0.24 mcg/mL (Day 5) • IV: 1.14 mcg/mL (healthy volunteers); 3.63 mcg/mL (hospitalized patients) • AUC • Oral (3-day regimen): 17.4 mcg·hr/mL • Oral (5-day regimen): 154.9 mcg·hr/mL • IV: 8.03 mcg·hr/mL (healthy volunteers); 9.6 mcg·hr/mL (hospitalized patients) • Effects of food • Oral suspension: When administered with food, peak plasma concentration increased by 56% and AUC unchanged • Tablets: No effect • Distribution • Protein bound: 51% (0.02 mcg/mL); 7% (2 mcg/mL) • Elimination • Clearance: 630 mL/min (single 500-mg oral and IV dose) • Half-life • Oral (3-day regimen): 71.8 hr • Oral (5-day regimen): 68.9 hr • Excretion • IV, 1st dose: 11%

				• IV, 5th dose: 14% • Oral: 6% (unchanged) • Biliary excretion is a major route of elimination for unchanged drug, following oral administration
22	NCT04324021	Human monoclonal antibody	•Drug: Emapalumab	• Absorption • Peak plasma concentration: 44 mcg/mL • Trough concentration: 25 mcg/mL • Steady-state: Achieved by 7th infusion • Distribution • Vd (wt 70 kg): 4.2 L (central); 5.6 L (peripheral) • Metabolism • Not characterized; like other protein therapeutics, expected to degrade into small peptides and amino acids via catabolic pathways • Elimination • Half-life: ~22 days (healthy volunteers); 2.5-18.9 days (HLH) • Clearance: ~0.007 L/hr (healthy volunteers); significantly influenced by IFN-gamma production in patients
23	NCT04322188	Anti-interleukin-6 (IL-6) monoclonal antibody	•Drug: Siltuximab	• Absorption • Peak plasma time: Occurs at end of IV infusion • Peak plasma concentration: 332 mcg/mL • Predose trough level: 84 mcg/mL • Distribution • Vd: 4.5 L (70 kg male) • Elimination • Half-life: 20.6 days • Clearance: 0.23 L/day
24	NCT04326790 NCT04322565 NCT04322682	Anti-mitotic	•Drug: Colchicine	**Absorption** Bioavailability: ~45% Onset: 18-24 hr Time to peak effect: 48-72 hr **Fasted** • Peak plasma concentration: 3 ng/mL (Mitigare); 2.16 ng/mL (Gloperba); 2.5-3.6 ng/mL (Colcrys) • Peak plasma time: 1 hr (Gloperba); 1.3-1.5 hr (Colcrys) • AUC: 19.9 hr·ng/mL (Gloperba) **Fed** • Peak plasma concentration: 1.68 ng/mL (Gloperba) • Peak plasma time: 2 hr (Gloperba) • AUC: 18.47 hr·ng/mL (Gloperba) **Distribution** Vd: ~1420L (Colcrys); ~5-8L/kg (Mitigare); 341-1150 L (Colcrys) Protein bound: 39% Colchicine crosses the placenta (plasma levels in the fetus reported to be ~15% of the maternal concentration) **Metabolism** Metabolized by P-gp and CYP3A4 May also be metabolized by glucuronidation Metabolites: Demethylated to 2 primary metabolites and 1 minor metabolite **Elimination** Half-life: 31 hr (Mitigare and Gloperba); 26.6-31.2 hr (Colcrys) Dialyzable: No (hemodialysis) Excretion: Urine (40-65%)
25	NCT04304313	PDE-5 Inhibitor	•Drug: Sildenafil Citrate	• Bioavailability: 40% • Peak plasma time: 30-120 min Metabolism • Metabolized in liver by CYP3A4 and (in minor amounts) CYP2C9 • Metabolites: N-desmethyl metabolite (active; possesses 50% of sildenafil's PDE-5-inhibiting activity)

				• Elimination • Half-life: Parent drug, 3-4 hr; active metabolite, 10-70 min • Excretion: Feces (80%), urine (13%)
26		Anti-inflammatory	•Drug: Hydrocortisone	• Absorption • Bioavailability: PO, 96% • Duration: Short-acting • Distribution • Protein bound: 90% • Vd: 34 L • Metabolism • Metabolized in tissues and liver • Metabolites: Glucuronide and sulfates (inactive) • Elimination • Half-life: Plasma, 1-2 hr; biologic, 8-12 hr • Excretion: Urine (mainly), feces (minimally)
27	NCT02735707	Fluoroquinolone antibiotics	•Drug: Levoflocxacin	Absorption • Well absorbed • Bioavailability: 99% • Peak serum time: 1-2 hr • Distribution • Cerebrospinal fluid (CSF) concentrations ~15% of serum levels; high concentrations achieved in prostate, gynecologic tissues, sinus, breast milk, saliva • Vd: 74-112 L • Metabolism • Limited metabolism in humans • Elimination • Half-life: 6-8 hr • Excretion: Urine (primarily as unchanged drug); after oral administration, 87% is recovered as unchanged drug in urine within 48 hr, and <4% is recovered in feces in 72 hr
28			•Drug: Ofloxacin	• Absorption • Well absorbed; food causes only minor alterations • Bioavailability: 98% • Distribution • Protein Bound: 32% • Vd: 2.4-3.5 L/kg • Elimination • Half-Life: 4-5 hr • Excretion: Urine (up to 80% unchanged; <5% metabolites); feces 4-8%
29		steroid hormones	•Drug: Estradiol	• Absorption • Readily absorbed through GI tract, skin, mucous membrane • Onset: PO, 2-4 weeks; transdermal, 4 hr • Duration: Estradiol valerate, 7-8 days; estradiol cypionate, 11 days • Distribution • Widely distributed • Protein bound: To globulin and albumin • Elimination • Half-life: 1.5-5 hr (IM); 4 hr (transdermal) • Excretion: Mainly in urine (as conjugates with small amount of unchanged drug); most estrogens are also excreted in bile and undergo enterohepatic recycling
30		Interleukin-6 (IL-6) receptor antagonist	•Drug: Sarilumab	• Absorption • Peak plasma time: 2-4 days • Peak plasma concentration: 20 mg/L (150 mg SC q2Weeks); 35.6 mg/L (200 mg SC q2Weeks) • Minimum plasma concentration: 6.35 mg/L (150 mg SC q2Weeks); 16.5 mg/L (200 mg SC q2Weeks) • AUC: 202 mg·day/L (150 mg SC q2Weeks); 395 mg·day/L (200 mg SC q2Weeks)

				• Steady state: Reached in 14-16 weeks • Distribution • Vd: 7.3 L • Metabolism • Expected to be degraded into small peptides and amino acids via catabolic pathways in the same manner as endogenous IgG • Elimination • Half-life: 10 days (200 mg q2wk); 8 days (150 mg q2wk) • Excretion: Monoclonal antibodies are not eliminated via renal or hepatic pathways
31	NCT04325061	Glucocorticoids and Anti-inflammation	•Drug: Dexamethasone (Dexamethasone acetate)	• Onset: Between a few minutes and several hours; dependent on indication and route of administration • Peak serum time: 8hr (IM); 1-2 hr (PO) • Distribution • Vd: 2 L/kg • Metabolism • Metabolized in liver • Elimination • Half-life: 1.8-3.5 hr (normal renal function) • Excretion: Urine (mainly), feces (minimally)
32	NCT04290858 NCT04305457 NCT04312243 NCT04306393 NCT03331445 NCT04290871	Vasodilator	• Drug: Nitric Oxide	• Excretion: Renal

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